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**Patent- og Varemærkestyrelsen**  
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Modtaget

**PROTEASE VARIANTS****FIELD OF THE INVENTION**

5 The present invention relates to variants of proteases belonging to the RP-II or C-component type, and methods for the construction of such variants with altered properties, such as stability (e.g. thermostability or storage stability),  $\text{Ca}^{2+}$  dependency, and pH dependent activity.

**BACKGROUND OF THE INVENTION**

10 Enzymes have been used within the detergent industry as part of washing formulations for more than 30 years. Proteases are from a commercial perspective the most relevant enzyme in such formulations, but other enzymes including lipases, amylases, cellulases, hemicellulases or mixtures of enzymes are also often used. Proteases are also used in other fields, such as production of dairy products, processing of  
15 hides, feed processing, etc.

To improve the cost and/or the performance of proteases there is an ongoing search for proteases with altered properties, such as increased activity at low temperatures, increased thermostability, increased specific activity at a given pH, altered  $\text{Ca}^{2+}$  dependency, increased stability in the presence of other detergent ingredients (e.g.  
20 bleach, surfactants etc.), modified specificity in respect of substrates, etc.

The search for proteases with altered properties includes both discovery of naturally occurring proteases, i.e. so called wild-type proteases but also alteration of well-known proteases by e.g. genetic manipulation of the nucleic acid sequence encoding said proteases. Knowledge of the relationship between the three-dimensional structure  
25 and the function of a protein has improved the ability to evaluate which areas of a protein to alter to affect a specific property of the protein.

One group of proteases, which has been indicated for use in detergents, food processing, feed processing is the RP-II proteases or C-component proteases belonging to the protease family S1B, glutamic-acid-specific endopeptidases. This family has  
30 till now only received relatively minor attention and has not been further grouped into different sub-groups. However, from the amino acid identities of isolated RP-II proteases it is evident that subgroups exist. Bacillus proteases of the RP-II type are serine proteases that in primary structure are similar to chymotrypsin.

The first description of a protease of the RP-II family of *Bacillus* proteases was in US Patent No. 4,266,031 (Tang et al., Novo Industri A/S), where it was designated Component C and tentatively (and incorrectly) characterised as not being a serine protease or metallo protease. Component C was considered a contaminant in the production of the *Bacillus licheniformis* alkaline protease, subtilisin Carlsberg.

In EP 369 817 (Omnigene Bioproducts, Inc.) the *B. subtilis* member of the RP-II family was identified by its amino acid and DNA sequences. The enzyme was again stated not to be a serine protease, and the family name RP-II designated (Residual Protease II). The enzyme was characterized further as a metallo protease by the inventors of EP 369 817 (Rufo et al., 1990, J. Bacteriol. 210:19-1023, and Sloma et al., 1990, J. Bacteriol. 172:1024-1029), designating the enzyme as mpr.

In WO 91/13553 (Novozymes A/S) the amino acid sequence of the C component was disclosed, stating that it is a serine protease specific for glutamic and aspartic acid, while EP 482 879 (Shionogi & Co. Ltd.) disclosed the enzyme and a DNA sequence encoding the C component from *B. licheniformis* ATCC No. 14580, naming the enzyme BLase. In EP 482 879 the protease is described as being specific for glutamic acid (see also Kakudo et al. "Purification, characterization, cloning, and expression of a glutamic acid-specific protease from *Bacillus licheniformis* ATCC 14580". J. Biol. Chem. 267:23782 (1992)).

In 1997 Okamoto et al. (Appl. Microbiol. Biotechnol. (1997) 48:27-33) found that the *B. subtilis* homologue of BLase, named BSase was identical to the above-mentioned enzyme, mpr/RP-II.

In 1999 Rebrikov et al. (Journal of Protein Chemistry, Vol. 18, No. 1, 1999) disclosed a Glu-specific protease from *B. intermedius* that also belongs to the RP-II family.

In WO 01/16285 a number of further RP-II protease were disclosed with DNA and amino acid sequences. These RP-II proteases were isolated from *B. pumilus*, *B. halmapalus* and *B. licheniformis*. WO 01/16285 also discloses a number of variants of RP-II proteases. These variants were based on various concepts relating to the primary structure of the RP-II proteases (amino acid sequences).

The homology matrix in Table 1 below clearly indicates that the RP-II proteases 1 to 8 are a distinct group of Glu-specific proteases that are clearly different from the other Glu-specific proteases in the Matrix

Table 1

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	100	99	97	60	55	55	47	59	46	45	45	47	49
2		100	99	60	60	59	50	61	50	44	45	46	52
3			100	60	57	54	47	60	47	45	45	44	49
4				100	94	92	68	57	44	38	40	42	47
5					100	91	59	54	44	42	40	43	45
6						100	63	53	39	42	46	41	45
7							100	48	41	41	40	36	44
8								100	50	45	46	46	54
9									100	63	53	55	49
10										100	53	56	52
11											100	78	54
12												100	53
13													100

In the matrix the sequences are identified by the patent publication in which first published or sequence database accession numbers.

1. *Bacillus* sp. JA96 glutamic-acid-specific endopeptidase, JA96, WO 01/16285
- 5 2. 1p3e *B. Intermedius*, glutamic-acid-specific endopeptidase, BIP, EMBL No. Y5136, Rebrikov et al., Journal of Protein Chemistry, Vol. 18, No. 1, 1999
3. *Bacillus* sp. BO32 glutamic-acid-specific endopeptidase, BO32, WO 01/16285
4. *Bacillus licheniformis*, BLC, WO 01/16285 (cf. US Patent No. 4,266,031)
5. *Bacillus* sp. CDJ31 glutamic-acid-specific endopeptidase, CDJ31, WO 01/16285
- 10 6. *Bacillus* sp. AC116 glutamic-acid-specific endopeptidase, AC116, WO 01/16285
7. mpr\_bacsu *Bacillus subtilis* serine protease, MPR, EP 369 817
8. *Bacillus* sp. AA513 glutamic-acid-specific endopeptidase, AA513, WO 01/16285
9. eta\_staau *Staphylococcus aureus* exfoliative toxin A (Lee et al. Sequence determination and comparison of the exfoliative toxin A and toxin B genes from *Staphylococcus aureus*; J. Bacteriol. 169:3904 (1987))
- 15 10. etb\_staau *Staphylococcus aureus* exfoliative toxin B (Jackson, M.P.; Iandolo, J.J.; Sequence of the exfoliative toxin B gene of *Staphylococcus aureus*; J. Bacteriol.

Accordingly, the object of the present invention is to provide a method for constructing RP-II proteases having altered properties, in particular to provide a method for constructing RP-II proteases having altered properties as described above.

Thus, in its broadest aspect, the present invention relates to a method for constructing a variant of a parent RP-II protease, wherein the variant has at least one altered property as compared to said parent RP-II protease, which method comprises:

i) analyzing the three-dimensional structure of the RP-II protease to identify, on the basis of an evaluation of structural considerations, at least one amino acid residue or at least one structural region of the RP-II protease, which is of relevance for altering said property;

ii) constructing a variant of the RP-II protease, which as compared to the parent RP-II protease, has been modified in the amino acid residue or structural part identified in i) so as to alter said property; and

iii) testing the resulting RP-II protease variant for said property.

Although it has been described in the following that modification of the parent RP-II protease in certain regions and/or positions is expected to confer a particular effect to the thus produced RP-II protease variant, it should be noted that modification of the parent RP-II protease in any of such regions may also give rise to any other of the above-mentioned effects. For example, any of the regions and/or positions mentioned as being of particular interest with respect to, e.g., improved thermostability, may also give rise to, e.g., higher activity at a lower pH, an altered pH optimum, or increased specific activity, such as increased peptidase activity.

Further aspects of the present invention relates to variants of a RP-II protease, the DNA encoding such variants and methods of preparing the variants. Still further aspects of the present invention relates to the use of the variants for various industrial purposes, in particular as an additive in detergent compositions. Other aspects of the present invention will be apparent from the below description as well as from the appended claims.

## **BRIEF DESCRIPTION OF DRAWINGS**

Fig. 1 provides a schematic structure of the RP-II protease from *Bacillus licheniformis*, BLC.

Fig. 2 shows a 3D structure based alignment of the wild type RP-II proteases 1 to 8 of Table 1.

Fig. 3 shows the BLC protease ribbon structure in black, with indication of active site residues, the bound peptide and the ion-binding site. The calcium ion is the sphere at the bottom of the Figure, the active site residues are in light grey and shown in stick model, and the bound peptide DAFE is in medium grey and shown in stick model.

5

## BRIEF DESCRIPTION OF APPENDICES

APPENDIX 1 provides the structural coordinates for the solved crystal 3D structure of the BLC RP-II protease, in the standard pdb format. The residues are numbered from 1-217, the calcium ion is numbered 301, and the DAFE substrate is numbered 401-404.

10

## DEFINITIONS

Prior to discussing this invention in further detail, the following terms and conventions will first be defined.

15

For a detailed description of the nomenclature of amino acids and nucleic acids and modifications introduced in a polypeptide or protein and especially in a RP-II protease by genetic manipulation, we refer to WO 01/16285 pages 5 to 15, hereby incorporated by reference.

20

The term "RP-II proteases" refers to a sub-group of serine protease, belonging to the protease family S1B, glutamic-acid-specific endopeptidases. Serine proteases or serine peptidases is a subgroup of proteases characterised by having a serine in the active site, which forms a covalent adduct with the substrate. Further the RP-II proteases (and the serine proteases) are characterised by having two active site amino acid residues apart from the serine, namely a histidine and an aspartic acid residue.

25

The RP-II proteases have a homology to the rest of the S1B protease family of around 50% (using the UWGCG version 8 software GAP program), or more preferred a homology higher than 55%. Table 1 demonstrate homologies between various S1B proteases. The RP-II proteases, nos. 1 to 8, are in Table 1 indicated in bold and the other S1B proteases, nos. 9 to 13, in bold italics. Table 1 shows that there is a clear distinction to the RP-II proteases from the other S1B proteases, but it is also clear that among the RP-II proteases there are subgroups. One subgroup comprises nos. 1, 2, and 3; and another subgroup comprises nos. 4, 5, and 6. The lengths of the listed RP-II proteases vary from 215 to 222 amino acid residues and experience within the subtilisin subgroups of subtilases indicates that such a variation in length probably has only

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little effect on the 3-dimensional structures of these and other RP-II protease subgroups.

## **PARENT**

5           The term "parent" is in the context of the present invention to be understood as a protein, which is modified to create a protein variant. The parent protein may be a naturally occurring (wild-type) polypeptide or it may be a variant thereof prepared by any suitable means. For instance, the parent protein may be a variant of a naturally occurring protein which has been modified by substitution, chemical modification, deletion or  
10   truncation of one or more amino acid residues, or by addition or insertion of one or more amino acid residues to the amino acid sequence, of a naturally-occurring polypeptide. Thus the term "parent RP-II protease" refers to a RP-II protease which is modified to create a RP-II protease variant.

## **15   VARIANT**

          The term "variant" is in the context of the present invention to be understood as a protein which has been modified as compared to a parent protein at one or more amino acid residues.

## **20   MODIFICATION**

          The term "modification(s)" or "modified" is in the context of the present invention to be understood as to include chemical modification of a protein as well as genetic manipulation of the DNA encoding a protein. The modification(s) may be replacement(s) of the amino acid side chain(s), substitution(s), deletion(s) and/or insertions in  
25   or at the amino acid(s) of interest. Thus the term "modified protein", e.g. "modified RP-II protease", is to be understood as a protein which contains modification(s) compared to a parent protein, e.g. RP-II protease.

## **HOMOLOGY**

30           "Homology" or "homologous to" is in the context of the present invention to be understood in its conventional meaning and the "homology" between two amino acid sequences should be determined by use of the "Similarity" parameter defined by the GAP program from the University of Wisconsin Genetics Computer Group (UWGCG)

package using default settings for alignment parameters, comparison matrix, gap and gap extension penalties. Default values for GAP penalties, i.e. GAP creation penalty of 3.0 and GAP extension penalty of 0.1 (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711). The method is also described in S.B. Needleman and C.D. Wunsch, Journal of Molecular Biology, 48, 443-445 (1970). Identities can be extracted from the same calculation. The homology between two amino acid sequences can also be determined by "identity" or "similarity" using the GAP routine of the UWGCG package version 9.1 with default setting for alignment parameters, comparison matrix, gap and gap extension penalties can also be applied using the following parameters: gap creation penalty = 8 and gap extension penalty = 8 and all other parameters kept at their default values. The output from the routine is besides the amino acid alignment the calculation of the "Percent Identity" and the "Similarity" between the two sequences. The numbers calculated using UWGCG package version 9.1 is slightly different from the version 8.

## NAMING OF RP-II PROTEASES

In describing the RP-II proteases of the invention the following abbreviations are used for ease of reference:

BLC = RP-II protease from *Bacillus licheniformis* (US Patent No. 4,266,031),  
 AA513 = RP-II protease from *Bacillus halmapalus* AA513 (WO 01/16285),  
 AC116 = RP-II protease from *Bacillus licheniformis* AC116 (WO 01/16285)  
 BO32 = RP-II protease from *Bacillus pumilus* BO32 (WO 01/16285),  
 CDJ31 = RP-II protease from *Bacillus licheniformis* CDJ31 (WO 01/16285),  
 JA96 = RP-II protease from *Bacillus pumilus* JA96 (WO 01/16285),  
 MPR = RP-II protease from *Bacillus subtilis* IS75 (EP 369 817 B1)  
 BIP = RP-II protease from *B. intermedius* (Rebrikov et al., Journal of Protein Chemistry, Vol. 18, No. 1, 1999)

## SEQUENCE LISTING

In the appended Sequence Listing the RP-II proteases are indicated as:  
 SEQ. ID. NO. 1 = BLC (DNA), SEQ. ID. NO. 2 = BLC (AA),  
 SEQ. ID. NO. 3 = AA513 (DNA), SEQ. ID. NO. 4 = AA513 (AA),  
 SEQ. ID. NO. 5 = AC116 (DNA), SEQ. ID. NO. 6 = AC116 (AA)

SEQ. ID. NO. 7 = BO32 (DNA), SEQ. ID. NO. 8 = BO32 (AA)  
 SEQ. ID. NO. 9 = CDJ31 (DNA), SEQ. ID. NO. 10 = CDJ31 (AA)  
 SEQ. ID. NO. 11 = JA96 (DNA), SEQ. ID. NO. 12 = JA96 (AA)  
 SEQ. ID. NO. 13 = BSMR (DNA), SEQ. ID. NO. 14 = BSMR (AA)  
 5 SEQ. ID. NO. 15 = BIP (DNA), SEQ. ID. NO. 16 = BIP (AA)

## POSITION

The term "position" is in the context of the present invention to be understood as the number of an amino acid residue in a peptide, polypeptide or protein when counting  
 10 from the N-terminal end of said peptide/polypeptide. The position numbers used here normally refer directly to different RP-II proteases.

The RP-II proteases are numbered individually according to each of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, and 16.

## 15 Corresponding position

The invention, however, is not limited to variants of these particular RP-II proteases but extends to parent proteases containing amino acid residues at positions which are "equivalent" to the particular identified residues in *Bacillus licheniformis* RP-II protease. In some preferred embodiment of the present invention, the parent protease is  
 20 JA96 or BIP RP-II protease and the substitutions are made at the equivalent amino acid residue positions in JA96 or BIP corresponding to those listed above.

A residue (amino acid) position of a RP-II protease is equivalent to a residue (position) of the *Bacillus licheniformis* RP-II protease if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or analogous to a specific  
 25 residue or portion of that residue in *Bacillus licheniformis* RP-II protease (i.e., having the same or similar functional capacity to combine, react, or interact chemically).

In order to establish homology to primary structure, the amino acid sequence of a precursor protease is directly compared to the *Bacillus licheniformis* RP-II protease, BLC, primary sequence by aligning the amino acid sequence of an isolated or parent  
 30 wild type enzyme with a suitable well-known enzyme of the same group or class of enzymes defines a frame of reference. This type of numbering was used in WO 01/16285. If nothing else is indicated herein, in the present instance the *Bacillus licheniformis* RP-II protease, first designated component C and therefore here abbreviated BLC, has been chosen as standard.

In order to establish homology to the tertiary structure (3D structure) of BLC, the 3D structure based alignment in Fig. 2 has been provided. By using this alignment the amino acid sequence of a precursor RP-II protease may be directly correlated to the *Bacillus licheniformis* RP-II protease, BLC, primary sequence. For a novel RP-II protease sequence, the (3D based) position corresponding to a position in BLC is found by

- i) identifying the RP-II protease from the alignment of Fig. 2 that is most homologous to the novel sequence,
- ii) aligning the novel sequence with the sequence identified to find the corresponding position in the RP-II protease from Fig. 2, and
- iii) establishing from Fig. 2 the corresponding position in BLC.

For comparison and finding the most homologous sequence the GAP program from GCG package as described below are used.

The alignment can as indicated above be obtained by the GAP routine of the GCG package version 8 to number the variants using the following parameters: gap creation penalty = 3 and gap extension penalty = 0.1 and all other parameters kept at their default values.

The alignment of Fig. 2 defines a number of deletions and insertions in relation to the sequence of BLC. In the alignment deletions are indicated by asterixes (\*) in the referenced sequence, and the referenced enzyme will be considered to have a gap at the position in question. Insertions are indicated by asterixes (\*) in the BLC sequence, and the positions in the referenced enzyme are given as the position number of the last amino acid residue where a corresponding amino acid residue exists in the standard enzyme with a lower case letter appended in alphabetical order, e.g. 82a, 82b, 82c, 82d, see Fig. 2.

In case the referenced enzyme contains a N- or C-terminal extension in comparison to BLC; an N-terminal extension is given the position number 0a, 0b, etc. in the direction of the N-terminal; and a C-terminal extension will be given either the position number of the C-terminal amino acid residue of BLC with a lower case letter appended in alphabetical order, or simply a continued consecutive numbering.

Thus for comparisons RP-II proteases are numbered by reference to the positions of the BLC RP-II protease (SEQ ID NO: 2) as provided in Fig. 2. The position is then indicated as "corresponding to BLC".

## DETAILED DESCRIPTION OF THE INVENTION

The inventors of the present invention have elucidated the three-dimensional structure of BLC, SEQ ID NO:2 by X-ray crystallography and found that there are several interesting features in the structure of this protease in comparison with the known structures of other proteases, such as the RP-II proteases. These features include both similarities and differences.

### RP-II proteases

As described above a RP-II protease is in the context of the present invention to be understood as a protease which has at least 50% homology to BLC (SEQ ID NO:2). In particular said protease may have at least 55% homology to BLC, i.e. to SEQ ID NO:2. The invention thus relates to variant RP-II proteases having at least 50% homology to BLC.

Specifically the variants of the invention may comprise RP-II proteases comprising a number of modifications or modifications in a number of positions ranging from at least one and up to 50, or from 1 to 45, or from 1 to 40, or from 1 to 35, or from 1 to 30, or from 1 to 25, or from 1 to 20, or from 1 to 15, or from 1 to 14, or from 1 to 13, or from 1 to 12, or from 1 to 11, or from 1 to 10, or from 1 to 9, or from 1 to 8, or from 1 to 7, or from 1 to 6, or from 1 to 5, or from 1 to 4, or from 1 to 3, or from 1 to 2 modifications or positions. Such modifications comprising substitutions, deletions and insertions in the indicated number or number of positions.

A RP-II protease variant of the present invention is encoded by an isolated polynucleotide, which nucleic acid sequence has at least 50% homology with the nucleic acid sequence shown in SEQ ID NO: 1, and where the polynucleotide encodes a variant RP-II protease in relation to a parent protease.

In a first embodiment of the present invention a RP-II protease suitable for the purpose described herein may be a RP-II protease homologous to the three-dimensional structure of BLC, i.e. it may be homologous to the three-dimensional structure defined by the structure coordinates in Appendix 1 by comprising the structural elements defined below.

It is well-known to a person skilled in the art that a set of structure coordinates for a protein or a portion thereof is a relative set of points that define a shape in three dimensions; it is possible that an entirely different set of coordinates defines an identical or a similar shape. Moreover, slight variations in the individual coordinates may have little or no effect on the overall shape.

These variations in coordinates may be generated because of mathematical manipulations of the structure coordinates. For example, the structure coordinates of Appendix 1 (BLC structure) may be manipulated by crystallographic permutations of the structure coordinates, fractionalization of the structure coordinates, integer additions or subtractions to sets of the structure coordinates, inversion of the structure coordinates or any combination of the above. Alternatively, said variations may be due to differences in the primary amino acid sequence.

When such variations are within an acceptable standard error as compared to the structure coordinates of Appendix 1 said three-dimensional structure is within the context of the present invention to be understood as being homologous to the structure of Appendix 1. The standard error may typically be measured as the root mean square deviation of e.g. conserved backbone residues, where the term "root mean square deviation" (RMS) means the square root of the arithmetic mean of the squares of the deviations from the mean.

It is also well-known to a person skilled in the art that within a group of proteins which have a homologous structure there may be variations in the three-dimensional structure in certain areas or domains of the structure, e.g. loops, which are not, or at least only of a small importance to the functional domains of the structure, but which may result in a big root mean square deviation of the conserved residue backbone atoms between said structures.

Thus it is well known that a set of structure coordinates is unique to the crystallised protein. No other three dimensional structure will have the exact same set of coordinates, be it a homologous structure or even the same protein crystallised in different manner. There are natural fluctuations in the coordinates. The overall structure and the inter-atomic relationship can be found to be similar. The similarity can be discussed in terms of root mean square deviation of each atom of a structure from each "homologous" atom of another structure. However, only identical proteins have the exact same number of atoms. Therefore, proteins having a similarity below 100% will often have a different number of atoms, and thus the root mean square deviation can not be calculated on all atoms, but only the ones that are considered "homologous". A precise description of the similarity based on the coordinates is thus difficult to describe and difficult to compute for homologous proteins. Regarding the present invention, similarities in 3D structure of different RP-II proteases can be described by the content of homologous structural elements, and/or the similarity in amino acid or DNA sequence

Examples of BLC like RP-II proteases include the BLC = RP-II protease from

*Bacillus licheniformis* (cf. US Patent No. 4,266,031), AA513 = RP-II protease from *Bacillus halmapalus* AA513 (NP000368), AC116 = RP-II protease from *Bacillus licheniformis* AC116 (NP000364), BO32 = RP-II protease from *Bacillus pumilus* BO32 (NP000366), CDJ31 = RP-II protease from *Bacillus licheniformis* CDJ31 (NP000365),  
 5 JA96 = RP-II protease from *Bacillus pumilus* JA96 (NP000367), MPR = RP-II protease from *Bacillus subtilis* IS75 (cf. EP 369 817 B1), BIP = RP-II protease from *B. intermedius* (EMBL No. Y5136, Rebrikov et al., Journal of Protein Chemistry, Vol. 18, No. 1, 1999)

Accordingly, a preferred embodiment of the present invention is a variant of a  
 10 parent RP-II protease or a RP-II protease variant which is at least 50% homologous to the sequence of SEQ ID NO 2 preferably at least 55%, preferably at least 65%, at least 70%, at least 74%, at least 80%, at least 83%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% homologous to the sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14 or 16.

15 A further embodiment of the invention is a RP-II protease variant comprising the following structural characteristics:

- a) two beta-barrel domains each comprising six long strands in antiparallel organisation,
- b) three alpha helices,
- 20 c) at least one ion-binding site,
- d) an active site comprising the amino acid residues His, Asp and Ser.

The potential ion binding site is defined as similar coordination or arrangement of the coordinates as in the 3D structure of BLC having one calcium ion coordinated by  
 25 the Ile 3 carbonyl atom O, the Ser 5 carbonyl atom O and bidentate by the Asp 161 Carboxyl acid group and the further coordination made by waters. The calcium may be substituted in the structure by water but then having the same coordination.

The RP-II protease variants of the present invention are encoded by isolated polynucleotides, which nucleic acid sequence has at least 45%, at least 50%, at least  
 30 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology with the nucleic acid sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15, and where the polynucleotide encodes a variant RP-II protease in relation to a parent protease.

35 Further the isolated nucleic acid sequence encoding a RP-II protease variant of

the invention hybridizes with a complementary strand of the nucleic acid sequence shown in SEQ ID NO: 1 preferably under low stringency conditions, at least under medium stringency conditions, at least under medium/high stringency conditions, at least under high stringency conditions, at least under very high stringency conditions.

5        Suitable experimental conditions for determining hybridization at low, medium, or high stringency between a nucleotide probe and a homologous DNA or RNA sequence involves presoaking of the filter containing the DNA fragments or RNA to hybridize in 5 x SSC (Sodium chloride/Sodium citrate, Sambrook et al. 1989) for 10 min, and prehybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution (Sambrook et al. 10        1989), 0.5 % SDS and 100 µg/ml of denatured sonicated salmon sperm DNA (Sambrook et al. 1989), followed by hybridization in the same solution containing a concentration of 10ng/ml of a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) *Anal. Biochem.* 132:6-13), <sup>32</sup>P-dCTP-labeled (specific activity > 1 x 10<sup>9</sup> cpm/µg) probe for 12 hours at ca. 45°C. The filter is then washed twice for 30 minutes in 2 x SSC, 0.5 % 15        SDS at least 55°C (low stringency), more preferably at least 60°C (medium stringency), still more preferably at least 65°C (medium/high stringency), even more preferably at least 70°C (high stringency), and even more preferably at least 75°C (very high stringency).

## 20        **Three-dimensional structure of RP-II proteases**

The BLC RP-II protease was used to elucidate the three-dimensional structure forming the basis for the present invention.

The structure of BLC was solved in accordance with the principle for x-ray crystallographic methods, for example, as given in X-Ray Structure Determination, 25        Stout, G.K. and Jensen, L.H., John Wiley & Sons, Inc. NY, 1989.

The structural coordinates for the solved crystal structure of BLC are given in standard PDB format (Protein Data Bank, Brookhaven National Laboratory, Brookhaven, CT) as set forth in Appendix 1. It is to be understood that Appendix 1 forms part of the present application. In the context of Appendix 1, the following abbreviations are 30        used: CA refers to c-alpha (carbon atoms) or to calcium ions, (however to avoid misunderstandings we normally use the full names "c-alpha atoms", "calcium" "Ca" or "ion" in the present specification). Amino acid residues are given in their standard three-letter code or the standard one-letter code. The structural coordinates in Appendix 1 contain the protease structure wherein the active serine was replaced by alanine and a com-

plex formed with the peptide DAFE (= Asp-Ala-Phe-Glu) as well as water molecules. The protease coordinates has a chain identification called A, whereas the peptide is called B, the calcium ion is called C, and the water is W. In the following the positions of the mentioned residues refer to the sequence of BLC as disclosed in SEQ ID NO: 2.

5 The overall structure of BLC falls into the S1 group of the proteases (MEROPS; <http://merops.sanger.ac.uk/>). The structure is a trypsin type of fold with two beta-barrel domains. The beta-barrel's each consists of six antiparallel beta-sheets folded into a beta-barrel. The topology can be described as S1-S2-S3-S6-S5-S4 for the strands in both beta-barrels. It is assumed that all the RP-II proteases fall within the same general overall structure.

10 The 3D structure of C-component serine protease from *Bacillus licheniformis* has 16 strands of which the 12 bigger strands compose the two beta-barrels; and 3 helices. The four very short strands are number 1, 5, 6 and 10 counting from the N-terminal and are composed of residue numbers 9-10, 50-51, 56-57 and 114-115. The other strands  
15 are residue numbers 22-26, 31-36, 41-44, 62-65, 77-83, 99-102, 126-131, 142-151, 156-159, 171-177, 182-192 and 201-205. One main helix C-terminal residue number 208-219. Two very small helices are composed of residues 86-90 and 106-110.

The active site consists of a triad involving the Ser in position 167, the His in position 47, and the Asp in position 96.

20 The 3D structure of BLC has one calcium ion coordinated by the carbonyl oxygen atom of Ile in position 3, the carbonyl oxygen atom of Ser in position 5, and bidentate by the Carboxylic acid group of Asp in position 161. Further coordinations are made by water molecules.

The calcium ion is placed in a distance from the CA atoms of the active site and  
25 Gly in position 168 as provided below:

Ser 167 CA atom to Ca ion: 16.07Å

His 47 CA atom to Ca ion: 24.27Å

Asp 96 CA atom to Ca ion: 23.72Å

Gly 168 CA atom to Ca ion: 19.20Å

30 The position of an ion-binding site can be defined by the distance to four specific atoms in the core structure. The distance from the ion-binding site to the c-alpha atoms of the three active site residues has been chosen. Throughout the RP-II proteases the residues Ser, His and Asp in the active site are highly conserved. In BLC they are  
35 Asp96, His47 and Ser167. The fourth distance chosen is the distance to the c-alpha

atom of the amino acid residue coming first after the active site serine residue in the sequence (herein after called "next to Ser"); in the 3D structure of BLC it is Gly168.

In a preferred embodiment of the present invention, the distance between the ion-binding site and i) Asp c-alpha atom is 22.50-24.00 Å, ii) His c-alpha atom is 23.25-25.25 Å, iii) Ser c-alpha atom is 15.00-17.00Å, iv) next to Ser c-alpha atom is 18.20-20.20 Å,

However these distances may vary from one RP-II protease to the other, and as described above, the ion binding site may also bind to a sodium ion. The present distances are given with a calcium ion in the structure. If a sodium ion was bound instead the distances would be shifted a little bit. Generally the distances can vary  $\pm 0.8\text{\AA}$ , preferably  $\pm 0.7\text{\AA}$ ,  $\pm 0.6\text{\AA}$ ,  $\pm 0.5\text{\AA}$ ,  $\pm 0.4\text{\AA}$ , or most preferably  $\pm 0.3\text{\AA}$ .

Further, in the RP-II proteases, the peptide structure circumscribing the ion-binding site is composed of the amino acid residues placed in positions 1-7, 159-162 and 143-145 with the coordinating atoms being the backbone carbonyl oxygen atom of residues I3, S5, D161 and water molecules.

3D structures of RP-II proteases can be modelled using the known structure of a related protease and general modelling tools as shown in Example 1. A prerequisite for obtaining a realistic 3D model structure is that the model is based on an adequate sequence homology higher than 50%, preferably higher than 55%, and even more preferred higher than 60% to the sequence of the protease for which the structure is known. RP-II Protease models can be constructed based on the 3D guided sequence alignments to BLC in Figure 2.

Therefore 3D structure models of RP-II proteases could in principle be made by using the modelling tools and the known 3D structure of the toxin A protease from *Staphylococcus aureus* from the Exf family of proteases (Cavarelli et al. (1997) The Structure of *Staphylococcus aureus* Epidermolytic Toxin A, an atypic serine protease, at 1.7 Å resolution, Structure, Vol. 5, p.813 (pdb name 1ARP).

If compared to the structure of the toxin A protease from *Staphylococcus aureus*, the structure of the RP-II proteases, as represented by BLC, can be divided into a "common protease" region, an "intermediate" region and a "nonhomologous" region.

The active site can be found in the common protease region, which is structurally closely related to the Toxin A structure. The common protease region is composed of residues 58, 70-83. The common protease region has an RMS lower than 1.2.

Outside the common protease region the structure of the RP-II protease BLC differs from the Toxin A structure to a greater extent.

The intermediate region consists of residues 14-28, 29-51, 94-104, 155-175. The intermediate region has an RMS bigger than 1.2 and less than 1.8. Any relationships between the three-dimensional structure and functionality based on modelling from the *S. aureus* 3D structure are potentially difficult to predict in this region of the RP-II proteases.

The common region and the intermediate region consist of the majority of the two central beta-barrels, especially the strands of the beta-barrels.

The nonhomologous region consists of residues 1-6, 7-13, 52-57, 59-69, 84-88, 89-93, 105-153. The nonhomologous region has a RMS higher than 1.5. Any relationships between the three-dimensional structure and functionality based on modelling from the *S. aureus* 3D structure are very difficult to predict in this region of the RP-II proteases.

Inferred structure-function relationships based on model building of a RP-II protease 3D structure on the 3D structure of *S. aureus* Toxin A would thus be very uncertain and speculative.

### **Homology building of RP-II proteases**

A model structure of a RP-II protease can be built using the BLC structure in Appendix 1, or a structure similar to the BLC structure comprising the structural elements (a) two beta-barrel domains each comprising six long strands in antiparallel organisation, (b) three alpha helices, (c) at least one low affinity ion-binding site, and (d) an active site comprising the amino acid residues His, Asp and Ser, or other 3D RP-II protease structures, e.g. established by X-ray structure determination, that may become available in the future, and the Homology™ program or a comparable program, e.g., Modeller™ (both from Molecular Simulations, Inc., San Diego, CA). The principle is to align the amino acid sequence of a protein for which the 3D structure is known with the amino acid sequence of a protein for which a model 3D structure has to be constructed. The structurally conserved regions can then be built on the basis of consensus sequences. In areas lacking homology, loop structures can be inserted, or sequences can be deleted with subsequent bonding of the necessary residues using, e.g., the program Homology. Subsequent relaxation and optimization of the structure should be done using either Homology or another molecular simulation program, e.g., CHARMM™ from Molecular Simulations.

## Methods for designing BLC and RP-II or S1B family protease variants

Comparisons of the molecular dynamics of different proteins can give a hint as to which domains are important or connected to certain properties pertained by each protein.

5 The present invention comprises a method of producing a variant of a parent BLC like RP-II protease, the variant having at least one altered property as compared to the parent BLC like RP-II protease, the method comprising:

- a) producing a model structure of the parent BLC like RP-II protease on the three-dimensional structure of BLC,
- 10 b) comparing the model three-dimensional structure of the parent BLC like RP-II protease to the BLC structure by superimposing the structures through matching the active residues CA, CB, C, O, and N atoms,
- c) identifying on the basis of the comparison in step a) at least one structural part of the parent BLC like RP-II protease, wherein an alteration in said structural part is predicted to result in an altered property;
- 15 d) modifying the nucleic acid sequence encoding the parent BLC like RP-II protease to produce a nucleic acid sequence encoding deletion or substitution of one or more amino acids at a position corresponding to said structural part, or an insertion of one or more amino acid residues in positions corresponding to said structural part;
- 20 e) expressing the modified nucleic acid sequence in a host cell to produce the variant RP-II protease;
- f) isolating the produced protease;
- g) purifying the isolated protease and
- 25 h) recovering the purified RP-II protease.

## Stability - alteration of ion-binding site

An ion-binding site is a significant feature of an enzyme. Therefore alterations of the amino acid residues close to the ion-binding site are likely to result in alterations of the stability of the enzyme. Especially modifications affecting the charge distribution and/or the electrostatic field strength at or in the vicinity of the site are important.

## Improved stability

Stabilisation of the ion-binding site of RP-II proteases may be obtained by modifications in positions close to the ion binding site.

Such modifications may comprise the substitution of a positively charged amino acid residue with a neutral or negatively charged residue, or the substitution of a neutral residue with a negatively charged residue or the deletion of a positively charged or neutral residue in positions close to the ion binding site.

Positions located at a distance of 10Å or less to the ion-binding site of BLC are: 1, 2, 3, 4, 5, 6, 7, 8, 143, 144, 145, 146, 158, 159, 160, 161, 162, 194, 199, 200, and 201. Especially positions 2, 3, 4, 5, 6, 7, 144, 159, 160, 161 located at a distance of 6 Å or less from the ion binding site are important.

Corresponding positions in other RP-II proteases may be identified using Fig. 2 herein.

The modifications D7E and D7Q in BLC are examples of suitable modifications in one of these positions.

### **Removal of ion-binding site in BLC**

By removing the ion-binding site it is possible to alter the enzymes dependency of calcium or other ions in the solution.

Removal of the Calcium site in BLC can be done by the substitutions H144R and/or D161R,K+H144Q,N (SEQ ID NO: 2). Similar modifications may be made in structurally corresponding residues in other RP-II proteases.

### **Alteration of thermostability**

A variant with improved stability (typically increased thermostability) may be obtained by modification of the mobility of identified regions, such as by introduction of disulfide bond(s), substitution with proline, alteration of hydrogen bond contact(s), altering charge distribution, introduction of salt bridge(s), filling in internal structural cavities with one or more amino acids with bulkier side groups (in e.g. regions which are structurally mobile), substitution of histidine residues with other amino acids, removal of a deamidation sites, or by helix capping.

### **Regions with increased mobility:**

The below indicated regions of BLC have an increased mobility in the crystal

structure of the enzyme, and it is presently believed that these regions can be responsible for stability or activity of BLC and the other RP-II proteases. Especially thermostabilisation may be obtained by altering the highly mobile regions. Generally, thermostability may be improved by making these regions less mobile. Improvements of the enzyme may be obtained by making modifications in the regions and positions identified below. Introducing e.g. larger residues or residues having more atoms in the side chain could increase the stability, or, e.g., introduction of residues having fewer atoms in the side chain could be important for the mobility and thus the activity profile of the enzyme. The regions can be found by analysing the B-factors taken from the coordinate file in Appendix 1, and/or from molecular dynamics calculations of the isotropic fluctuations. These can be obtained by using the program CHARMM from MSI (Molecular Simulations Inc.).

Molecular dynamics simulation at 300K and 400K of BLC reveals the following highly mobile regions:

26-31, 50-55, 89-91, and 193-198, and 4-5, 11-12, 26-31, 50-55, 69-70, 89-91, 178-183, 195-199 and 216-221, respectively.

It is contemplated that modifications in these regions may influence the thermostability of RP-II proteases. Modifications are preferably made in the regions 26-31 (26, 27, 28, 29, 30, 31); 89-91 (89, 90, 91); 216-221 (216, 217, 218, 219, 220, 221), and especially in BLC the substitutions G30A and G91A. Similar modifications may be made in structurally corresponding residues in other RP-II proteases.

Also B-factors (see "in X-Ray Structure Determination, Stout, G.K. and Jensen, L.H., John Wiley & Sons, Inc. NY, 1989") from crystallographic data indicate the following more mobile regions in the BLC (RP-II protease) structure:

51-56, (i.e. 51, 52, 53, 54, 55, 56)

88-94, (i.e. 88, 89, 90, 91, 92, 93, 94)

118-122 (i.e. 118, 119, 120, 121, 122)

173-183 (i.e. 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183)

It is contemplated that modifications in these regions may influence the thermostability of RP-II proteases. Modifications are preferably made in the regions 51-56 and 118-122.

#### Disulfide bonds:

A RP-II protease variant of the present invention with improved stability, e.g.

thermostability, as compared to the parent RP-II protease may be obtained by introducing new inter-domain or intra-domain bonds to provide a more rigid and stable structure, such as by establishing inter- or intra-domain disulfide bridges. This is done by introducing cysteines in appropriate positions in the RP-II molecule by substitution(s) or insertion(s).

According to the guidelines mentioned above the below mentioned amino acid residues identified in the amino acid sequence of SEQ ID NO: 2 are contemplated as being suitable for cysteine replacement. With one or more of these substitutions with cysteine, disulfide bridges may form in a variant of BLC. A stabilising disulfide bridge may be constructed through the substitutions: S145C and T128C

### Surface charge distribution

A variant with improved stability (typically improved thermostability or storage stability) as compared to the parent RP-II protease may be obtained by changing the surface charge distribution of the RP-II protease. For example, when the pH is lowered to about 5 or below, histidine residues typically become positively charged and, consequently, unfavorable electrostatic interactions on the protein surface may occur. By engineering the surface charge of the RP-II protease one may avoid such unfavorable electrostatic interactions that in turn may lead to a higher stability of the RP-II protease.

Charged amino acid residues are (a) positively charged: Lys, Arg, His (pH<5), Tyr (pH>9) and Cys (pH>10??) and (b) negatively charged: Asp and Glu.

The surface charge distribution may be modified by (a) removing charged residues from the surface through deletion of a charged residue or substituting an uncharged residue for a charged residue, (b) adding charged residues to the surface through insertion of a charged residue or substituting a charged residue for an uncharged residue, or (c) by reverting the charge at a residue through substituting a positively charged residue for a negatively charged residue or substituting a negatively charged residue for a positively charged residue.

Therefore, a further aspect of the present invention relates to a method for constructing a variant of a parent RP-II protease having a modified surface charge distribution, the method comprising:

- a) identifying, on the surface of the parent RP-II protease, at least one charged amino acid residue;
- b) modifying the charged residue identified in step (a) through deletion or substitu-

- tion with an uncharged amino acid residue;
- c) optionally repeating steps a) and b) recursively;
  - d) preparing the variant resulting from steps a) - c);
  - e) testing the stability of said variant; and
  - 5 f) optionally repeating steps a) - e) recursively; and
  - g) selecting a RP-II protease variant having increased stability as compared to the parent RP-II protease.

As will be understood by the skilled person it may also, in some cases, be  
10 advantageous to substitute an uncharged amino acid residue with an amino acid  
residue bearing a charge or, alternatively, it may in some cases be advantageous to  
substitute an amino acid residue bearing a charge with an amino acid residue bearing a  
charge of opposite sign. Thus, the above-mentioned method may be employed by the  
skilled person also for these purposes. In the case of substituting an uncharged amino  
15 acid residue with an amino acid residue bearing a charge the above-mentioned method  
may be employed the only difference being steps a) and b) which will then read:

- a) identifying, on the surface of the parent RP-II protease, at least one position be-  
ing occupied by an uncharged amino acid residue;
- b) modifying the charge in that position by substituting the uncharged amino acid  
20 residue with a charged amino acid residue or by insertion of a charged amino  
acid residue at the position.

Also in the case of changing the sign of an amino acid residue present on the  
surface of the RP-II protease the above method may be employed. Again, compared to  
25 the above method, the only difference being steps a) and b) which, in this case, read:

- a) identifying, on the surface of the parent RP-II protease, at least one charged  
amino acid residue;
- b) substituting the charged amino acid residue identified in step (a) with an amino  
acid residue having an opposite charge.

30

In order to determine the amino acid residues of a protease, which are present  
on the surface of the enzyme, the surface accessible area are measured using the  
DSSP program (Kabsch and Sander, *Biopolymers* (1983), 22, 2577-2637). All residues  
having a surface accessibility higher than 0, 0.10, 0.20, 0.30, 0.35, 0.40, 0.45, 0.50,  
35 0.55 or 0.60 are regarded a surface residue.

An amino acid residue found on the surface of BLC using the above method is T109 and it is contemplated that the substitutions T109R, K, H are of particular interest.

Similar substitutions may be introduced in equivalent positions of other RP-II proteases.

5 For the purpose of providing RP-II protease variants exhibiting improved wash performance it is possible to modify the pI of the RP-II protease through modification of the surface charge as indicated in WO 91/00345 (Novozymes A/S) and/or WO 99/20771 (Genencor International, Inc.)

Especially changing the pI of the RP-II protease is of interest

10 Changes in BLC:

T109R, K, H

Q143R, K, H

E209Q, N

D7N, S, T

15 Q174R, K, H

N216R, K, H

Y17R, K, H

Y95R, K, H

20 Corresponding modifications may be performed in corresponding positions of other RP-II proteases.

### **Substitution with proline residues**

Improved thermostability of a RP-II protease can be obtained by subjecting the RP-II protease in question to analysis for secondary structure, identifying residues in the  
25 RP-II protease having dihedral angles  $\phi$  (phi) and  $\psi$  (psi) confined to the intervals  $[-90^\circ < \phi < -40^\circ$  and  $-180^\circ < \psi < 180^\circ]$ , preferably the intervals  $[-90^\circ < \phi < -40^\circ$  and  $120^\circ < \psi < 180^\circ]$  or  $[-90^\circ < \phi < -40^\circ$  and  $-50^\circ < \psi < 10^\circ]$  and excluding residues located in regions in which the RP-II protease is characterized by possessing  $\alpha$ -helical or  $\beta$ -sheet structure.

30 After the dihedral angles  $\phi$  (phi) and  $\psi$  (psi) for the amino acids have been calculated, based on the atomic structure in the crystalline RP-II proteases, it is possible to select position(s) which has/have dihedral phi and psi angles favourable for substitution with a proline residue. The aliphatic side chain of proline residues is bonded covalently to the nitrogen atom of the peptide group. The resulting cyclic five-membered ring consequently imposes a rigid constraint on the rotation about the N-C $_{\alpha}$  bond of the peptide backbone

and simultaneously prevents the formation of hydrogen bonding to the backbone N-atom.

For these structural reasons, proline residues are generally not compatible with  $\alpha$ -helical and  $\beta$ -sheet secondary conformations.

If a proline residue is not already at the identified position(s), the naturally occurring amino acid residue is substituted with a proline residue, preferably by site directed mutagenesis applied on a gene encoding the RP-II protease in question.

In the group of BLC- like proteases proline residues can be introduced at positions 18, 115, 185, 269 and 293. Accordingly, a preferred BLC variant has one or more of the substitutions: T60P, S221P, G193P, and V194P.

#### Alteration of activity:

Amino acid residues at a distance of less than 10Å from the active site residues are most likely to influence the specificity and activity of the RP-II proteases, therefore variants comprising modifications in positions 1, 8, 22-35 (22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35), 42-58 (42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58), 82-100 (82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100), 129-135 (129, 130, 131, 132, 133, 134, 135), 141-142, 153-156 (153, 154, 155, 156), 158, 161-171 (161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171), 188-193 (188, 189, 190, 191, 192, 193), 195, 201-207 (201, 202, 203, 204, 205, 206, 207), 210, 213-214, 217 may provide a change in activity and/or specificity of the RP-II protease variant.

#### Substrate binding site

The substrate binding site is identified by the residues in contact with a substrate model, such as the DAFE. The 3D structure coordinates of the BLC protease with DAFE bound in the active site can be found in Appendix 1. Without being limited to any theory, it is presently believed that binding between a substrate and an enzyme is supported by favorable interactions found within a sphere 10 Å from the substrate molecule, in particular within a sphere of 6 Å from the substrate molecule. Examples of such favorable bonds are hydrogen bonds, strong electrostatic interaction and/or hydrophobic interactions.

The following residues of the BLC protease (SEQ ID NO:1), are within a distance of 10Å from the peptide DAFE and thus believed to be involved in interactions with said substrate: 1, 2, 3, 8, 25, 29, 30, 31, 32, 33, 34, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53,

90, 91, 92, 93, 94, 95, 96, 97, 129, 131, 132, 133, 134, 135, 155, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 171, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 200 and 204.

5 The following residues of the BLC protease (SEQ ID NO: 1), are within a distance of 6Å from the peptide DAFE and thus believed to be involved in interactions with said substrate: 1, 2, 31, 32, 47, 48, 88, 91, 93, 96, 162, 163, 164, 165, 166, 167, 168, 190, 191, 192, 193, 194, 195, and 201.

### **Helix capping:**

10 For the RP-II proteases helix capping may be obtained by modifying the position structurally corresponding to position 221 in BLC, and specifically in BLC by the modification A221N,T

### **Removal of deamidation sites**

15 For the RP-II proteases, removal of deamidation sites may be obtained by modifying the positions structurally corresponding to positions 213, 216, and 222 of BLC, and specifically in BLC by the modifications.

N213A,C,D,E,F,G,H,I,K,L,P,Q,R,S,T,V,Y,M,W preferably N213L,T,S

N216A,C,D,E,F,G,H,I,K,L,P,Q,R,S,T,V,Y,M,W preferably N216L,T,S

20 N222A,C,D,E,F,G,H,I,K,L,P,Q,R,S,T,V,Y,M,W preferably N222L,T,S

### **Combined modifications**

25 The present invention also encompasses any of the above mentioned RP-II protease variants in combination with any other modification to the amino acid sequence thereof. Especially combinations with other modifications known in the art to provide improved properties to the enzyme are envisaged. Such modifications to be combined with any of the above indicated modifications are exemplified in the following.

### **Removal of critical oxidation sites**

30 In order to increase the stability of the RP-II protease it may be advantageous to substitute or delete critical oxidation sites, such as methionines, with other amino acid residues which are not subject to oxidation.

Accordingly, in a further embodiment the present invention relates to an RP-II protease variant, in which one or more amino acid residues susceptible to oxidation, especially methionine residues exposed to the surface of the molecule, is/are deleted or replaced with another amino acid residue less susceptible to oxidation. The amino acid residue less susceptible to oxidation may for instance be selected from the group consisting of A, E, N, Q, I, L, S and K.

Specific such variants comprises at least one of the deletions or substitutions M36{\*,S,A,N,Q,K}; M160{\*,S,A,N,Q,K} of the BLC protease; M144{\*,S,A,N,Q,K} of the AC116 and CDJ31 proteases; M67{\*,S,A,N,Q,K}, M79{\*,S,A,N,Q,K}, M137{\*,S,A,N,Q,K}, M144{\*,S,A,N,Q,K}, and M171{\*,S,A,N,Q,K} of the BO32, BIP and JA96 proteases; M159{\*,S,A,N,Q,K} of the BO32 protease; M81{\*,S,A,N,Q,K}, and M141{\*,S,A,N,Q,K} in the MPR protease; and M17{\*,S,A,N,Q,K}, M67{\*,S,A,N,Q,K}, M144{\*,S,A,N,Q,K}, M160{\*,S,A,N,Q,K}, M186{\*,S,A,N,Q,K}, and M217{\*,S,A,N,Q,K} of the AA513 protease (positions are indicated in relation to the BLC protease as indicated in Fig. 2).

#### Modification of Asn-Gly sequences in the protease

It is known that at alkaline pH, the side chain of Asn may interact with the NH group of a sequential neighboring amino acid to form an isoAsp residue where the backbone goes through the Asp side chain. This will leave the backbone more vulnerable to proteolysis. The deamidation is much more likely to occur if the residue that follows is a Gly. Changing the Asn in front of the Gly or the Gly will prevent this from happening and thus improve the stability, especially as concerns thermo- and storage stability.

The invention consequently further relates to an RP-II protease variant, in which either or both residues of any of the Asn-Gly sequence appearing in the amino acid sequence of the parent RP-II protease is/are deleted or substituted with a residue of a different amino acid.

The Asn and/or Gly residue may, for instance, be substituted with a residue of an amino acid selected from the group consisting of A, Q, S, P, T and Y.

More specifically, any of the Asn or Gly residues of the Asn-Gly occupying positions 68-69, 182-183 and/or 192-193 of the BLC protease; positions 68-69 and/or 192-193 of the AC116 and CDJ-31 proteases, positions 45-46, 74-75, 196-197, and/or

201-202 of the BO32, JA96 and BIP proteases, positions 68-69, 103-104 and/or 192-196 of the MPR protease; and positions 90-91 and/or 201-202 of the AA513 protease, may be deleted or substituted with a residue of an amino acid selected from the group consisting of A, Q, S, P, T and Y. (positions are indicated in relation to the BLC protease as indicated in Fig. 2)

Specific variants of BLC are:

N68{\* ,A,Q,S,P,T,Y};      G69{\* ,A,Q,S,P,T,Y}

N68{\* ,A,Q,S,P,T,Y}+G69{\* ,A,Q,S,P,T,Y}

N182{\* ,A,Q,S,P,T,Y};      G183{\* ,A,Q,S,P,T,Y}

10 N182{\* ,A,Q,S,P,T,Y}+G183{\* ,A,Q,S,P,T,Y}

N192{\* ,A,Q,S,P,T,Y};      G193{\* ,A,Q,S,P,T,Y}

N192{\* ,A,Q,S,P,T,Y}+G193{\* ,A,Q,S,P,T,Y}

and combinations thereof.

Specific variants of the AC116 and CDJ-31 proteases are:

15 N68{\* ,A,Q,S,P,T,Y};      G69{\* ,A,Q,S,P,T,Y}

N68{\* ,A,Q,S,P,T,Y}+G69{\* ,A,Q,S,P,T,Y}

N192{\* ,A,Q,S,P,T,Y};      G193{\* ,A,Q,S,P,T,Y}

N192{\* ,A,Q,S,P,T,Y}+G193{\* ,A,Q,S,P,T,Y}

N68{\* ,A,Q,S,P,T,Y}+N192{\* ,A,Q,S,P,T,Y}

20 and combinations thereof.

Specific variants of BO32, JA96 and BIP proteases are:

N45{\* ,A,Q,S,P,T,Y};      G46{\* ,A,Q,S,P,T,Y}

N45{\* ,A,Q,S,P,T,Y}+G46{\* ,A,Q,S,P,T,Y}

N74{\* ,A,Q,S,P,T,Y};        G75{\* ,A,Q,S,P,T,Y}

N74{\* ,A,Q,S,P,T,Y}+G75{\* ,A,Q,S,P,T,Y}

N196{\* ,A,Q,S,P,T,Y};        G197{\* ,A,Q,S,P,T,Y}

N196{\* ,A,Q,S,P,T,Y}+G197{\* ,A,Q,S,P,T,Y}

5    N201{\* ,A,Q,S,P,T,Y};        G202{\* ,A,Q,S,P,T,Y}

N201{\* ,A,Q,S,P,T,Y} + G202{\* ,A,Q,S,P,T,Y}

N45{\* ,A,Q,S,P,T,Y}+N74{\* ,A,Q,S,P,T,Y}

N45{\* ,A,Q,S,P,T,Y}+N196{\* ,A,Q,S,P,T,Y}

N45{\* ,A,Q,S,P,T,Y}+N201{\* ,A,Q,S,P,T,Y}

10   N74{\* ,A,Q,S,P,T,Y}+N196{\* ,A,Q,S,P,T,Y}

N74{\* ,A,Q,S,P,T,Y}+N201{\* ,A,Q,S,P,T,Y}

N196{\* ,A,Q,S,P,T,Y}+N201{\* ,A,Q,S,P,T,Y}

N45{\* ,A,Q,S,P,T,Y}+N74{\* ,A,Q,S,P,T,Y}+N196{\* ,A,Q,S,P,T,Y}

N45{\* ,A,Q,S,P,T,Y}+N74{\* ,A,Q,S,P,T,Y}+N201{\* ,A,Q,S,P,T,Y}

15   N45{\* ,A,Q,S,P,T,Y}+N196{\* ,A,Q,S,P,T,Y}+N201{\* ,A,Q,S,P,T,Y}

N74{\* ,A,Q,S,P,T,Y}+N196{\* ,A,Q,S,P,T,Y}+N201{\* ,A,Q,S,P,T,Y}

N45{\* ,A,Q,S,P,T,Y}+N74{\* ,A,Q,S,P,T,Y}+N196{\* ,A,Q,S,P,T,Y}+N201{\* ,A,Q,S,P,T,Y}

and combinations thereof.

Specific variants of AA513 are:

20   N90{\* ,A,Q,S,P,T,Y};        G91{\* ,A,Q,S,P,T,Y}

N90{\* ,A,Q,S,P,T,Y}+G91{\* ,A,Q,S,P,T,Y}

N201{\* ,A,Q,S,P,T,Y};        G202{\* ,A,Q,S,P,T,Y}

N201{\*,A,Q,S,P,T,Y}+G202{\*,A,Q,S,P,T,Y}

N90{\*,A,Q,S,P,T,Y}+N201{\*,A,Q,S,P,T,Y}

and combinations thereof.

Specific variants of MPR are:

5 N68{\*,A,Q,S,P,T,Y}; G69{\*,A,Q,S,P,T,Y}

N68{\*,A,Q,S,P,T,Y}+G69{\*,A,Q,S,P,T,Y}

N103{\*,A,Q,S,P,T,Y}; G104{\*,A,Q,S,P,T,Y}

N103{\*,A,Q,S,P,T,Y}+G104{\*,A,Q,S,P,T,Y}

N192{\*,A,Q,S,P,T,Y}; G196{\*,A,Q,S,P,T,Y}

10 N192{\*,A,Q,S,P,T,Y}+G196{\*,A,Q,S,P,T,Y}

N68{\*,A,Q,S,P,T,Y}+N103{\*,A,Q,S,P,T,Y}

N68{\*,A,Q,S,P,T,Y}+N192{\*,A,Q,S,P,T,Y}

N103{\*,A,Q,S,P,T,Y}+N192{\*,A,Q,S,P,T,Y}

N68{\*,A,Q,S,P,T,Y}+N103{\*,A,Q,S,P,T,Y}+N192{\*,A,Q,S,P,T,Y}

15 and combinations thereof.

### **Removal of autoproteolysis sites**

According to a further aspect of the invention autoproteolysis sites may be removed by changing the amino acids at an autoproteolysis site. Since the RP-II proteases cleaves at Glu and Asp residues it is preferred to modify such residues of a parent RP-II protease having the same or a similar specificity, preferably by substituting with any other amino acid except Glu.

The parent RP-II proteases are mostly specific towards Glu and to a minor extent towards Asp residues. Therefore the modification of the parent (trypsin-like) RP-II protease may preferably be made by changing Glu to another amino acid residue (including Asp). Experiments have indicated that the substitution of Ala for Glu or Asp

provides good results.

Glu and Asp residue are in the BLC, CDJ31 and AC116 proteases found in positions E101, E152, E173, E209, D6, D51, D96, D135, D161, and D212. BLC has a further Glu in position E104 and Asp in D7.

5        Specific BLC, CDJ31 and AC116 variants are thus E101A, E152A, E173A, E209A, D6A, D51A, D135A, D161A, D212A, and double, triple, quadruple, etc. combinations thereof. Further specific BLC variants are E104A and D7A.

In JA96, BO32 and BIP Glu and Asp are found at positions E81, E143, E151, E209, D5, D6, D69, D96, D103, D135, D152, D161, and D173.

10       Specific JA96, BO32 and BIP variants are thus E81A, E143A, E151A, E202A, D5A, D6A, D69A, D96A, D103A, D135A, D152A, D161A, D173A, and double, triple, quadruple, etc. combinations thereof.

In MPR Glu and Asp are found at positions E7, E89a, E152, D6, D54, D92, D96, D135, D144, D161, D177 and D209

15       Specific MPR variants are thus E7A, E89aA, E152A, D6A, D54A, D92A, D96A, D135A, D144A, D161A, D177A and D209A, and double, triple, quadruple, etc. combinations thereof.

In AA513 Glu and Asp are found at positions E26, E55, E94, E117, E123, E137b, E199, D40, D96, D103b, D103d, D135, D149, D154, D161, D184 and D209

20       Specific AA513 variants are thus E26A, E55A, E94A, E117A, E123A, E137bA, E199A, D40A, D96A, D103bA, D103dA, D135A, D149A, D154A, D161A, D184A and D209A, and double, triple, quadruple, etc. combinations thereof.

Corresponding variants are easily identified in any other RP-II protease.

25       Alternatively autoproteolysis can be prevented by changing the amino acid residue occupying the 1st and/or 2nd position following the Glu or Asp residue in question to Pro. For instance, this may in BLC, CDJ31 and AC116 be done in the positions 174 and/or 175 as follows:

Q174P; S175P; Q174P+S175P

or in a similar manner in JA96, BO32 or BIP at positions 152 and/or 153 as D152P; T153P; or D152P+T153P.

Corresponding variants are easily identified in these and any other RP-II protease.

### **Modification of tryptophan residues**

5 In order to stabilize the protein it may be advantageous to replace or delete tryptophan residues at the surface of the protein, e.g., as described in US 5,118,623. The tryptophan residues may advantageously be substituted for F, T, Q or G. Thus, in a further embodiment the invention relates to an RP-II variant comprising one or more of the following substitutions:

#### 10 BLC and AC116:

W35{F,T,Q,G}; W88{F,T,Q,G}; W142{F,T,Q,G}; W217{F,T,Q,G}

#### CDJ31:

W142{F,T,Q,G}; W217{F,T,Q,G};

#### BO32, JA96 and BIP:

15 W142{F,T,Q,G};

#### AA513:

W30{F,T,Q,G}; W72{F,T,Q,G}; W142{F,T,Q,G}

#### MPR:

W57{F,T,Q,G}; W88{F,T,Q,G}; W112{F,T,Q,G}; W142{F,T,Q,G}; W217{F,T,Q,G}

### 20 **Modification of tyrosines**

In relation to wash performance it has been found that the modification of certain tyrosine residues to phenylalanine provides an improved wash performance. Without being bound by any specific theory, it is believed that titration of these Tyr residues in the alkaline wash liquor has negative effects that are alleviated by replacing the Tyr residues  
25 with other residues, especially Phe or Trp, particularly Phe.

In the BLC, AC116 and CDJ31 parent RP-II proteases, the following tyrosine

residues may be modified:

19, 50, 72, 74, 82, 95, 97, 112, 115, 117, 132, 154, 163, 195, 200. In BLC and CDJ31 the tyrosines in positions 17 and 158 may also be modified , and in AC116 and CDJ31 the tyrosines in position 172

5           Examples of specific variants comprise one or more of the following substitutions:

Y17{F,W}, Y19{F,W}, Y50{F,W}, Y72{F,W}, Y74{F,W}, Y82{F,W}, Y88{F,W}, Y95{F,W}, Y97{F,W}, Y112{F,W}, Y115{F,W}, Y117{F,W}, Y132{F,W}, Y154{F,W}, Y158{F,W}, Y163{F,W}, Y172{F,W}, Y195{F,W}, Y200{F,W}

10           In the JA96, BO32 and BIP parent RP-II proteases, the following tyrosine residues may be modified:

19, 24, 50, 57, 64, 83, 88, 95, 112, 132, 157, 158, 195, 216

Examples of specific JA96, BO32 and BIP variants comprises one or more of the following substitutions:

15   Y19{F,W}, Y24{F,W}, Y50{F,W}, Y57{F,W}, Y64{F,W}, Y83{F,W}, Y88{F,W}, Y95{F,W}, Y112{F,W}, Y132{F,W}, Y157{F,W}, Y158{F,W}, Y195{F,W} and Y216{F,W}

In the AA513 parent RP-II protease, the following tyrosine residues may be modified:

24, 74, 77, 84, 88, 97, 130, 132, 158, 163, 193a

20           Examples of specific AA513 variants comprises one or more of the following substitutions:

Y24{F,W}, Y74{F,W}, Y77{F,W}, Y84{F,W}, Y88{F,W}, Y97{F,W}, Y130{F,W}, Y132{F,W}, Y158{F,W}, Y163{F,W}, Y193A{F,W}

25           In the MPR parent RP-II protease, the following tyrosine residues may be modified:

19, 28a, 30, 50, 72, 74, 77, 83, 95, 97, 113, 115, 154, 158, 163, 172, 175, 200, 216

Examples of specific MPR variants comprises one or more of the following sub-

stitutions:

Y19{F,W}, Y28Ad{F,W}, Y30{F,W}, Y50{F,W}, Y72{F,W}, Y74{F,W}, Y77{F,W}, Y83{F,W},  
Y95{F,W}, Y97{F,W}, Y113{F,W}, 115{F,W}, Y154{F,W}, Y158{F,W}, Y163{F,W},  
Y172{F,W}, Y175{F,W}, Y200{F,W}, Y216{F,W}

5 **Other modifications for combination**

Examples of specific BLC variants comprises one or more of the following substitutions:

E152{A,R,K,G}

E173A

10 E209A

E152G+G164R

**METHODS OF PREPARING RP-II PROTEASE VARIANTS**

15 The RP-II protease variants of the present invention may be produced by any known method within the art. The invention also relates to polynucleotides encoding the RP-II protease variants of the present invention, DNA constructs comprising such polynucleotides and host cells comprising such constructs or polynucleotides.

20 In general natural occurring proteins may be produced by culturing the organism expressing the protein and subsequently purifying the protein, or recombinantly by cloning a polynucleotide, e.g. genomic DNA or cDNA, encoding the protein into an expression vector, introducing said expression vector into a host cell, culturing the host cell and purifying the expressed protein.

25 **site-directed mutagenesis**

Typically protein variants may be produced by site-directed mutagenesis of the gene encoding a parent protein, introduction of the mutated gene into an expression vector, host cell etc. The gene encoding the parent protein may be cloned from a strain producing the polypeptide or from an expression library, i.e. it may be isolated from ge-

omic DNA or prepared from cDNA, or a combination thereof. The gene may even be a fully synthetically produced gene.

In general standard procedures for cloning of genes and/or introducing mutations (random and/or site directed) into said genes may be used in order to obtain a parent RP-II protease, or RP-II protease variant of the invention. For further description of suitable techniques reference is made to Molecular cloning: A laboratory manual (Sambrook et al. (1989), Cold Spring Harbor lab., Cold Spring Harbor, NY; Ausubel, F. M. et al. (eds.)); Current protocols in Molecular Biology (John Wiley and Sons, 1995; Harwood, C. R., and Cutting, S. M. (eds.)); Molecular Biological Methods for Bacillus (John Wiley and Sons, 1990); DNA Cloning: A Practical Approach, Volumes I and II (D.N. Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds (1985)); Transcription And Translation (B.D. Hames & S.J. Higgins, eds. (1984)); Animal Cell Culture (R.I. Freshney, ed. (1986)); Immobilized Cells And Enzymes ( IRL Press, (1986)); A Practical Guide To Molecular Cloning (B. Perbal, (1984)) and WO 96/34946.

#### **Localized and region specific random mutagenesis**

Random mutagenesis is suitably performed either as localized or region-specific random mutagenesis in at least three parts of the gene translating to the amino acid sequence shown in question, or within the whole gene.

The random mutagenesis of a DNA sequence encoding a parent RP-II protease may be conveniently performed by use of any method known in the art.

In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent RP-II protease wherein the variant exhibits an altered property, such as increased thermostability, increased stability at low pH and at low calcium concentration, relative to the parent RP-II protease, the method comprising:

- a) subjecting a DNA sequence encoding the parent protease to localized or region-specific random mutagenesis,
- b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and
- c) screening for host cells expressing a RP-II protease variant which has an altered property relative to the parent RP-II protease.

Step (a) of the above method of the invention is preferably performed using doped primers.

When the mutagenesis is performed by the use of an oligonucleotide, the oligonucleotide may be doped or spiked with the three non-parent nucleotides during the synthesis of the oligonucleotide at the positions that are to be changed. The doping or spiking may be done so that codons for unwanted amino acids are avoided. The doped or spiked oligonucleotide can be incorporated into the DNA encoding the RP-II protease by any published technique, using, e.g., PCR, LCR or any DNA polymerase and ligase as deemed appropriate.

Preferably, the doping is carried out using "constant random doping", in which the percentage of wild-type and modification in each position is predefined. Furthermore, the doping may be directed toward a preference for the introduction of certain nucleotides, and thereby a preference for the introduction of one or more specific amino acid residues. The doping may be made, e.g., so as to allow for the introduction of 90% wild type and 10% modifications in each position. An additional consideration in the choice of a doping scheme is based on genetic as well as protein-structural constraints. The doping scheme may be made by using the DOPE program which, *inter alia*, ensures that introduction of stop codons is avoided (L.J. Jensen et al. *Nucleic Acid Research*, 26, 697-702 (1998)).

The DNA sequence to be mutagenized may conveniently be present in a genomic or cDNA library prepared from an organism expressing the parent RP-II protease. Alternatively, the DNA sequence may be present on a suitable vector such as a plasmid or a bacteriophage, which as such may be incubated with or otherwise exposed to the mutagenizing agent. The DNA to be mutagenized may also be present in a host cell either by being integrated in the genome of said cell or by being present on a vector harboured in the cell. Finally, the DNA to be mutagenized may be in isolated form. It will be understood that the DNA sequence to be subjected to random mutagenesis is preferably a cDNA or a genomic DNA sequence.

In some cases it may be convenient to amplify the mutated DNA sequence prior to performing the expression step b) or the screening step c). Such amplification may be performed in accordance with methods known in the art, the presently preferred method being PCR-generated amplification using oligonucleotide primers prepared on the basis of the DNA or amino acid sequence of the parent enzyme.

Subsequent to the incubation with or exposure to the mutagenizing agent, the mutated DNA is expressed by culturing a suitable host cell carrying the DNA sequence under conditions allowing expression to take place. The host cell used for this purpose may be one which has been transformed with the mutated DNA sequence, optionally

present on a vector, or one which was carried the DNA sequence encoding the parent enzyme during the mutagenesis treatment. Examples of suitable host cells are the following: gram positive bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amy-*  
 5 *loliuefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus megaterium*, *Bacillus thuringiensis*, *Streptomyces lividans* or *Streptomyces murinus*; and gram negative bacteria such as *E. coli*.

The mutated DNA sequence may further comprise a DNA sequence encoding functions permitting expression of the mutated DNA sequence.

10

### Localised random mutagenesis

The random mutagenesis may be advantageously localised to a part of the parent RP-II protease in question. This may, e.g., be advantageous when certain regions of the enzyme have been identified to be of particular importance for a given property  
 15 of the enzyme, and when modified are expected to result in a variant having improved properties. Such regions may normally be identified when the tertiary structure of the parent enzyme has been elucidated and related to the function of the enzyme.

The localised or region-specific, random mutagenesis is conveniently performed by use of PCR generated mutagenesis techniques as described above or any other  
 20 suitable technique known in the art. Alternatively, the DNA sequence encoding the part of the DNA sequence to be modified may be isolated, e.g., by insertion into a suitable vector, and said part may be subsequently subjected to mutagenesis by use of any of the mutagenesis methods discussed above.

### 25 General method for localised random mutagenesis by use of the DOPE program

The localised random mutagenesis may be carried out by the following steps:

1. Select regions of interest for modification in the parent enzyme
2. Decide on mutation sites and non-mutated sites in the selected region
3. Decide on which kind of mutations should be carried out, e.g. with re-  
 30 spect to the desired stability and/or performance of the variant to be constructed
4. Select structurally based mutations
5. Adjust the residues selected in step 3 with regard to step 4.
6. Analyse by use of a suitable dope algorithm the nucleotide distribu-

tion.

7. If necessary, adjust the wanted residues to genetic code realism, e.g. taking into account constraints resulting from the genetic code, e.g. in order to avoid introduction of stop codons; the skilled person will be aware that some codon combinations cannot be used in practice and will need to be adapted
8. Make primers
9. Perform localised random mutagenesis by use of the primers
10. Select resulting RP-II protease variants by screening for the desired improved properties.

Suitable dope algorithms for use in step 6 are well known in the art. One such algorithm is described by Tomandl, D. et al., 1997, Journal of Computer-Aided Molecular Design 11:29-38. Another algorithm is DOPE (Jensen, LJ, Andersen, KV, Svendsen, A, and Kretzschmar, T (1998) Nucleic Acids Research 26:697-702).

### Expression vectors

A recombinant expression vector comprising a nucleic acid sequence encoding a RP-II protease variant of the invention may be any vector that may conveniently be subjected to recombinant DNA procedures and which may bring about the expression of the nucleic acid sequence.

The choice of vector will often depend on the host cell into which it is to be introduced. Examples of a suitable vector include a linear or closed circular plasmid or a virus. The vector may be an autonomously replicating vector, i.e., a vector which exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, e.g., a plasmid, an extra-chromosomal element, a mini chromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, pACYC184, pUB110, pE194, pTA1060, and pAMB1. Examples of origin of replications for use in a yeast host cell are the 2 micron origin of replication, the combination of CEN6 and ARS4, and the combination of CEN3 and ARS1. The origin of replication may be one having a mutation which makes it function as temperature-sensitive in the host cell (see, e.g., Ehrlich, 1978, Proceedings of the National Academy of Sciences USA 75:1433).

Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Vectors which are integrated into the genome of the host cell may contain any nucleic acid sequence enabling integration into the genome; in particular it may contain nucleic acid sequences facilitating integration into the genome by homologous or non-homologous recombination. The vector system may be a single vector, e.g. plasmid or virus, or two or more vectors, e.g. plasmids or virus', which together contain the total DNA to be introduced into the genome of the host cell, or a transposon.

The vector may in particular be an expression vector in which the DNA sequence encoding the RP-II protease variant of the invention is operably linked to additional segments or control sequences required for transcription of the DNA. The term, "operably linked" indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in a promoter and proceeds through the DNA sequence encoding the RP-II protease variant. Additional segments or control sequences include a promoter, a polyadenylation sequence, a propeptide sequence, a signal sequence and a transcription terminator. At a minimum the control sequences include a promoter and transcriptional and translational stop signals.

The promoter may be any DNA sequence that shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell.

Examples of suitable promoters for use in bacterial host cells include the promoter of the *Bacillus subtilis* levansucrase gene (sacB), the *Bacillus stearothermophilus* maltogenic amylase gene (amyM), the *Bacillus licheniformis* alpha-amylase gene (amyL), the *Bacillus amyloliquefaciens* alpha-amylase gene (amyQ), the *Bacillus subtilis* alkaline protease gene, or the *Bacillus pumilus* xylosidase gene, the *Bacillus amyloliquefaciens* BAN amylase gene, the *Bacillus licheniformis* penicillinase gene (penP), the *Bacillus subtilis* xylA and xylB genes, and the prokaryotic beta-lactamase gene (Villa-Kamaroff et al., 1978, Proceedings of the National Academy of Sciences USA 75:3727-3731). Other examples include the phage Lambda P<sub>R</sub> or P<sub>L</sub> promoters or the *E. coli* lac, trp or tac promoters or the *Streptomyces coelicolor* agarase gene (dagA). Further promoters are described in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; and in Sambrook et al., 1989, supra.

Examples of suitable promoters for use in a filamentous fungal host cell are promoters obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Rhi-*

*zomucor miehei* aspartic proteinase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (glaA), *Rhizomucor miehei* lipase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Aspergillus nidulans* acetamidase, *Fusarium oxysporum* trypsin-like protease (as described in U.S. Patent No. 4,288,627, which is incorporated herein by reference), and hybrids thereof. Particularly preferred promoters for use in filamentous fungal host cells are the TAKA amylase, NA2-tpi (a hybrid of the promoters from the genes encoding *Aspergillus niger* neutral (-amylase and *Aspergillus oryzae* triose phosphate isomerase), and glaA promoters. Further suitable promoters for use in filamentous fungus host cells are the ADH3 promoter (McKnight et al., The EMBO J. 4 (1985), 2093 - 2099) or the tpiA promoter.

Examples of suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255 (1980), 12073 - 12080; Alber and Kawasaki, J. Mol. Appl. Gen. 1 (1982), 419 - 434) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals (Hollaender et al, eds.), Plenum Press, New York, 1982), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., Nature 304 (1983), 652 - 654) promoters.

Further useful promoters are obtained from the *Saccharomyces cerevisiae* enolase (ENO-1) gene, the *Saccharomyces cerevisiae* galactokinase gene (GAL1), the *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase genes (ADH2/GAP), and the *Saccharomyces cerevisiae* 3-phosphoglycerate kinase gene. Other useful promoters for yeast host cells are described by Romanos et al., 1992, Yeast 8:423-488. In a mammalian host cell, useful promoters include viral promoters such as those from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus, and bovine papilloma virus (BPV).

Examples of suitable promoters for use in mammalian cells are the SV40 promoter (Subramani et al., Mol. Cell Biol. 1 (1981), 854 -864), the MT-1 (metallothionein gene) promoter (Palmiter et al., Science 222 (1983), 809 - 814) or the adenovirus 2 major late promoter.

An example of a suitable promoter for use in insect cells is the polyhedrin promoter (US 4,745,051; Vasuvedan et al., FEBS Lett. 311, (1992) 7 - 11), the P10 promoter (J.M. Vlak et al., J. Gen. Virology 69, 1988, pp. 765-776), the Autographa californica polyhedrosis virus basic protein promoter (EP 397 485), the baculovirus immediate early gene 1 promoter (US 5,155,037; US 5,162,222), or the baculovirus 39K delayed-early gene promoter (US 5,155,037; US 5,162,222).

The DNA sequence encoding a RP-II protease variant of the invention may also, if necessary, be operably connected to a suitable terminator.

The recombinant vector of the invention may further comprise a DNA sequence enabling the vector to replicate in the host cell in question.

5       The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, or a gene encoding resistance to e.g. antibiotics like ampicillin, kanamycin, chloramphenicol, erythromycin, tetracycline, spectinomycin, neomycin, hygromycin, methotrexate, or resistance to heavy metals, virus or herbicides, or which provides for prototrophy or auxotrophs. Examples of bacterial selectable markers are the *dal* genes from *Bacillus subtilis* or *Bacillus licheniformis*, resistance. A frequently used mammalian marker is the dihydrofolate reductase gene (DHFR). Suitable markers for yeast host cells are ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. A selectable marker for use in a filamentous fungal host cell may be selected from the group including, but not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hygB* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenylyltransferase), *trpC* (anthranilate synthase), and glutamate resistance markers, as well as equivalents from other species. Particularly, for use in an *Aspergillus* cell are the *amdS* and *pyrG* markers of *Aspergillus nidulans* or *Aspergillus oryzae* and the *bar* marker of *Streptomyces hygroscopicus*. Furthermore, selection may be accomplished by co-transformation, e.g., as described in WO 91/17243, where the selectable marker is on a separate vector.

To direct a RP-II protease variant of the present invention into the secretory pathway of the host cells, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) may be provided in the recombinant vector. The secretory signal sequence is joined to the DNA sequence encoding the enzyme in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the enzyme. The secretory signal sequence may be that normally associated with the enzyme or may be from a gene encoding another secreted protein.

30       The procedures used to ligate the DNA sequences coding for the present enzyme, the promoter and optionally the terminator and/or secretory signal sequence, respectively, or to assemble these sequences by suitable PCR amplification schemes, and to insert them into suitable vectors containing the information necessary for replication or integration, are well known to persons skilled in the art (cf., for instance, Sam-

brook et al.).

More than one copy of a nucleic acid sequence encoding an enzyme of the present invention may be inserted into the host cell to amplify expression of the nucleic acid sequence. Stable amplification of the nucleic acid sequence can be obtained by  
 5 integrating at least one additional copy of the sequence into the host cell genome using methods well known in the art and selecting for transformants.

The nucleic acid constructs of the present invention may also comprise one or more nucleic acid sequences which encode one or more factors that are advantageous in the expression of the polypeptide, e.g., an activator (e.g., a trans-acting factor), a  
 10 chaperone, and a processing protease. Any factor that is functional in the host cell of choice may be used in the present invention. The nucleic acids encoding one or more of these factors are not necessarily in tandem with the nucleic acid sequence encoding the polypeptide.

## 15 **Host cells**

The DNA sequence encoding a RP-II protease variant of the present invention may be either homologous or heterologous to the host cell into which it is introduced. If homologous to the host cell, i.e. produced by the host cell in nature, it will typically be operably connected to another promoter sequence or, if applicable, another secretory  
 20 signal sequence and/or terminator sequence than in its natural environment. The term "homologous" is intended to include a DNA sequence encoding an enzyme native to the host organism in question. The term "heterologous" is intended to include a DNA sequence not expressed by the host cell in nature. Thus, the DNA sequence may be from another organism, or it may be a synthetic sequence.

25 The host cell into which the DNA construct or the recombinant vector of the invention is introduced may be any cell that is capable of producing the present RP-II protease variants, such as prokaryotes, e.g. bacteria or eukaryotes, such as fungal cells, e.g. yeasts or filamentous fungi, insect cells, plant cells or mammalian cells.

Examples of bacterial host cells which, on cultivation, are capable of producing  
 30 the RP-II protease variants of the invention are gram-positive bacteria such as strains of *Bacillus*, e.g. strains of *B. subtilis*, *B. licheniformis*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. amyloliquefaciens*, *B. coagulans*, *B. circulans*, *B. lautus*, *B. megaterium* or *B. thuringiensis*, or strains of *Streptomyces*, such as *S. lividans* or *S. murinus*, or gram-negative bacteria such as *Escherichia coli* or *Pseudomo-*

*nas* sp.

The transformation of the bacteria may be effected by protoplast transformation, electroporation, conjugation, or by using competent cells in a manner known per se (cf. Sambrook et al., supra).

When expressing the RP-II protease variant in bacteria such as *E. coli*, the enzyme may be retained in the cytoplasm, typically as insoluble granules (known as inclusion bodies), or it may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed and the granules are recovered and denatured after which the enzyme is refolded by diluting the denaturing agent. In the latter case, the enzyme may be recovered from the periplasmic space by disrupting the cells, e.g. by sonication or osmotic shock, to release the contents of the periplasmic space and recovering the enzyme.

When expressing the RP-II protease variant in gram-positive bacteria such as *Bacillus* or *Streptomyces* strains, the enzyme may be retained in the cytoplasm, or it may be directed to the extracellular medium by a bacterial secretion sequence. In the latter case, the enzyme may be recovered from the medium as described below.

Examples of host yeast cells include cells of a species of *Candida*, *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Pichia*, *Hansehula*, or *Yarrowia*. In a particular embodiment, the yeast host cell is a *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis* or *Saccharomyces oviformis* cell. Other useful yeast host cells are a *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Hansehula polymorpha*, *Pichia pastoris*, *Yarrowia lipolytica*, *Schizosaccharomyces pombe*, *Ustilgo maylis*, *Candida maltose*, *Pichia guillermondii* and *Pichia methanolio* cell (cf. Gleeson et al., J. Gen. Microbiol. 132, 1986, pp. 3459-3465; US 4,882,279 and US 4,879,231). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in Biology and Activities of Yeast (Skinner, F.A., Passmore, S.M., and Davenport, R.R., eds, Soc. App. Bacteriol. Symposium Series No. 9, 1980. The biology of yeast and manipulation of yeast genetics are well known in the art (see, e.g., Biochemistry and Genetics of Yeast, Bacil, M., Horecker, B.J., and Stopani, A.O.M., editors, 2nd edition, 1987; The Yeasts, Rose, A.H., and Harrison, J.S., editors, 2nd edition, 1987; and The Molecular Biology of the Yeast *Saccharomyces*, Strathern et al., editors, 1981). Yeast may be transformed using the procedures described by Becker and Guarente, In Abelson, J.N. and Simon, M.I., editors, Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, Volume 194,

pp 182-187, Academic Press, Inc., New York; Ito et al., 1983, Journal of Bacteriology 153:163; and Hinnen et al., 1978, Proceedings of the National Academy of Sciences USA 75:1920.

Examples of filamentous fungal cells include filamentous forms of the subdivision Eumycota and Oomycota (as defined by Hawksworth et al., 1995, supra), in particular it may be of the a cell of a species of *Acremonium*, such as *A. chrysogenum*, *Aspergillus*, such as *A. awamori*, *A. foetidus*, *A. japonicus*, *A. niger*, *A. nidulans* or *A. oryzae*, *Fusarium*, such as *F. bactridioides*, *F. cerealis*, *F. crookwellense*, *F. culmorum*, *F. graminearum*, *F. graminum*, *F. heterosporum*, *F. negundi*, *F. reticulatum*, *F. roseum*, *F. sambucinum*, *F. sarcochroum*, *F. sulphureum*, *F. trichothecioides* or *F. oxysporum*, *Humicola*, such as *H. insolens* or *H. lanuginosa*, *Mucor*, such as *M. miehei*, *Myceliophthora*, such as *M. thermophilum*, *Neurospora*, such as *N. crassa*, *Penicillium*, such as *P. purpurogenum*, *Thielavia*, such as *T. terrestris*, *Tolypocladium*, or *Trichoderma*, such as *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. reesei* or *T. viride*, or a teleomorph or synonym thereof. The use of *Aspergillus* spp. for the expression of proteins is described in, e.g., EP 272 277, EP 230 023.

Examples of insect cells include a *Lepidoptera* cell line, such as *Spodoptera frugiperda* cells or *Trichoplusia ni* cells (cf. US 5,077,214). Culture conditions may suitably be as described in WO 89/01029 or WO 89/01028. Transformation of insect cells and production of heterologous polypeptides therein may be performed as described in US 4,745,051; US 4, 775, 624; US 4,879,236; US 5,155,037; US 5,162,222; EP 397,485).

Examples of mammalian cells include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, COS cells, or any number of other immortalized cell lines available, e.g., from the American Type Culture Collection. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159 (1982), 601 - 621; Southern and Berg, J. Mol. Appl. Genet. 1 (1982), 327 - 341; Loyter et al., Proc. Natl. Acad. Sci. USA 79 (1982), 422 - 426; Wigler et al., Cell 14 (1978), 725; Corsaro and Pearson, Somatic Cell Genetics 7 (1981), 603; Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., N.Y., 1987, Hawley-Nelson et al., Focus 15 (1993), 73; Ciccarone et al., Focus 15 (1993), 80; Graham and van der Eb, Virology 52 (1973), 456; and Neumann et al., EMBO J. 1 (1982), 841 - 845. Mammalian cells may be transfected by direct uptake using the calcium phosphate precipitation method of Graham and Van der Eb (1978, Virology 52:546).

## Methods for expression and isolation of proteins

To express an enzyme of the present invention the above mentioned host cells transformed or transfected with a vector comprising a nucleic acid sequence encoding an enzyme of the present invention are typically cultured in a suitable nutrient medium under conditions permitting the production of the desired molecules, after which these are recovered from the cells, or the culture broth.

The medium used to culture the host cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The media may be prepared using procedures known in the art (see, e.g., references for bacteria and yeast; Bennett, J.W. and LaSure, L., editors, *More Gene Manipulations in Fungi*, Academic Press, CA, 1991).

If the enzymes of the present invention are secreted into the nutrient medium, they may be recovered directly from the medium. If they are not secreted, they may be recovered from cell lysates. The enzymes of the present invention may be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like, dependent on the enzyme in question.

The enzymes of the invention may be detected using methods known in the art that are specific for these proteins. These detection methods include use of specific antibodies, formation of a product, or disappearance of a substrate. For example, an enzyme assay may be used to determine the activity of the molecule. Procedures for determining various kinds of activity are known in the art.

The enzymes of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing (IEF), differential solubility (e.g., ammonium sulfate precipitation), or extraction (see, e.g., *Protein Purification*, J-C Janson and Lars Ryden, editors, VCH Publishers, New York, 1989).

When an expression vector comprising a DNA sequence encoding an enzyme of the present invention is transformed/transfected into a heterologous host cell it is possible to enable heterologous recombinant production of the enzyme. An advantage of using a heterologous host cell is that it is possible to make a highly purified enzyme composition, characterized in being free from homologous impurities, which are often present when a protein or peptide is expressed in a homologous host cell. In this context homologous impurities mean any impurity (e.g. other polypeptides than the enzyme of the invention) which originates from the homologous cell where the enzyme of the invention is originally obtained from.

## DETERGENT APPLICATIONS

The enzyme of the invention may be added to and thus become a component of a detergent composition.

The detergent composition of the invention may for example be formulated as a hand or machine laundry detergent composition including a laundry additive composition suitable for pre-treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations, or be formulated for hand or machine dishwashing operations.

In a specific aspect, the invention provides a detergent additive comprising the enzyme of the invention. The detergent additive as well as the detergent composition may comprise one or more other enzymes such as a protease, a lipase, a cutinase, an amylase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, e.g., a laccase, and/or a peroxidase.

In general the properties of the chosen enzyme(s) should be compatible with the selected detergent, (i.e. pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzyme(s) should be present in effective amounts.

### Proteases:

Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. The protease may be a serine protease or a metallo protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin

Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270 and WO 94/25583.

5 Examples of useful proteases are the variants described in WO 92/19729, WO 98/20115, WO 98/20116, and WO 98/34946, especially the variants with substitutions in one or more of the following positions: 27, 36, 57, 76, 87, 97, 101, 104, 120, 123, 167, 170, 194, 206, 218, 222, 224, 235 and 274.

Preferred commercially available protease enzymes include Alcalase™, Savinase™, Primase™, Duralase™, Esperase™, and Kannase™ (Novozymes A/S),  
10 Maxatase™, Maxacal™, Maxapem™, Properase™, Purafect™, Purafect OxP™, FN2™, and FN3™ (Genencor International Inc.).

#### Lipases:

Suitable lipases include those of bacterial or fungal origin. Chemically modified  
15 or protein engineered mutants are included. Examples of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g. from *H. lanuginosa* (*T. lanuginosus*) as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580, a *Pseudomonas* lipase, e.g. from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 2 18 272), *P. cepacia* (EP 3 31 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*,  
20 *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), a *Bacillus* lipase, e.g. from *B. subtilis* (Dartois et al. (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).

Other examples are lipase variants such as those described in WO 92/05249,  
25 WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202.

Preferred commercially available lipase enzymes include Lipolase™ and Lipolase Ultra™ (Novozymes A/S).

#### 30 Amylases:

Suitable amylases ( $\alpha$  and/or  $\beta$ ) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example,  $\alpha$ -amylases obtained from *Bacillus*, e.g. a special strain of *B. licheniformis*, described in more detail in GB 1,296,839.

Examples of useful amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

5       Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™ and BAN™ (Novozymes A/S), Rapidase™ and Purastar™ (from Genencor International Inc.).

#### Cellulases:

10       Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g. the fungal cellulases produced from *Humicola insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US  
15 5,691,178, US 5,776,757 and WO 89/09259.

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046,  
20 US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and PCT/DK98/00299.

Commercially available cellulases include Celluzyme™, and Carezyme™ (Novozymes A/S), Clazinase™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

#### 25   Peroxidases/Oxidases:

Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, e.g. from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257.

30       Commercially available peroxidases include Guardzyme™ (Novozymes A/S).

The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, i.e. a separate  
35 additive or a combined additive, can be formulated e.g. as a granulate, a liquid, a

slurry, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, or slurries.

Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethylene glycol, PEG) with mean molar weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The detergent composition of the invention may be in any convenient form, e.g., a bar, a tablet, a powder, a granule, a paste or a liquid. A liquid detergent may be aqueous, typically containing up to 70 % water and 0-30 % organic solvent, or non-aqueous.

The detergent composition comprises one or more surfactants, which may be non-ionic including semi-polar and/or anionic and/or cationic and/or zwitterionic. The surfactants are typically present at a level of from 0.1% to 60% by weight.

When included therein the detergent will usually contain from about 1% to about 40% of an anionic surfactant such as linear alkylbenzenesulfonate, alpha-olefinsulfonate, alkyl sulfate (fatty alcohol sulfate), alcohol ethoxysulfate, secondary alkylsulfonate, alpha-sulfo fatty acid methyl ester, alkyl- or alkenylsuccinic acid or soap.

When included therein the detergent will usually contain from about 0.2% to about 40% of a non-ionic surfactant such as alcohol ethoxylate, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide, or N-acyl N-alkyl derivatives of glucosamine ("glucamides").

The detergent may contain 0-65 % of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, carbonate, citrate, nitrilotriacetic acid, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst).

The detergent may comprise one or more polymers. Examples are carboxy-

methylcellulose, poly(vinylpyrrolidone), poly (ethylene glycol), poly(vinyl alcohol), poly(vinylpyridine-N-oxide), poly(vinylimidazole), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

5 The detergent may contain a bleaching system which may comprise a  $H_2O_2$  source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetythylenediamine or nonanoyloxybenzenesulfonate. Alternatively, the bleaching system may comprise peroxyacids of e.g. the amide, imide, or sulfone type.

10 The enzyme(s) of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in e.g. WO 92/19709 and WO 92/19708.

15 The detergent may also contain other conventional detergent ingredients such as e.g. fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil redeposition agents, dyes, bactericides, optical brighteners, hydrotropes, tarnish inhibitors, or perfumes.

20 It is at present contemplated that in the detergent compositions any enzyme, in particular the enzyme of the invention, may be added in an amount corresponding to 0.01-100 mg of enzyme protein per litre of wash liquor, preferably 0.05-5 mg of enzyme protein per litre of wash liquor, in particular 0.1-1 mg of enzyme protein per litre of wash liquor.

25 The enzyme of the invention may additionally be incorporated in the detergent formulations disclosed in WO 97/07202 which is hereby incorporated as reference.

## FOOD PROCESSING APPLICATIONS

30 The RP-II protease variants of the present invention may also be used in the processing of food, especially in the field of dairy products, such as milk, cream and cheese, but also in the processing of meat and vegetables.

## FEED PROCESSING APPLICATION

The RP-II protease variants of the present invention may also be used in the processing of feed for cattle, poultry, and pigs and especially for pet food.

## TREATMENT OF HIDES

The RP-II protease variants of the invention may also be used for the treatment of hides.

5

## MATERIALS AND METHODS

### Strains:

*B. subtilis* DN1885: Disclosed in WO 01/16285

10

### Plasmids:

pNM1003: Disclosed in WO 01/16285

pSX222: Disclosed in WO 96/34946

pNM1008: See Example 2

15

### Method for producing a protease variant

The present invention provides a method of producing an isolated enzyme according to the invention, wherein a suitable host cell, which has been transformed with a DNA sequence encoding the enzyme, is cultured under conditions permitting the production of the enzyme, and the resulting enzyme is recovered from the culture.

20

When an expression vector comprising a DNA sequence encoding the enzyme is transformed into a heterologous host cell it is possible to enable heterologous recombinant production of the enzyme of the invention. Thereby it is possible to make a highly purified RP-II protease composition, characterized in being free from homologous impurities.

25

The medium used to culture the transformed host cells may be any conventional medium suitable for growing the host cells in question. The expressed RP-II protease may conveniently be secreted into the culture medium and may be recovered therefrom by well-known procedures including separating the cells from the medium by centrifugation or filtration, precipitating proteinaceous components of the medium by means of a salt such as ammonium sulfate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

30

## Proteolytic Activity

Enzyme activity can be measured using the PNA assay using succinyl-alanine-alanine-proline-glutamicacid-paranitroaniline as a substrate. The principle of the PNA assay is described in the Journal of American Oil Chemists Society, Rothgeb, T.M.,  
 5 Goodlander, B.D., Garrison, P.H., and Smith, L.A., (1988).

## Textiles

Standard textile pieces are obtained from EMPA St. Gallen, Lerchfeldstrasse 5, CH-9014 St. Gallen, Switzerland. Especially type EMPA 116 (cotton textile stained with  
 10 blood, milk and ink) and EMPA 117 (polyester/cotton textile stained with blood, milk and ink).

## EXAMPLE 1

### Modelling RP-II proteases from the 3D structure of BLC

15 The overall homology of *Bacillus licheniformis* protease BCL to other RP-II proteases is high. The similarity between the different RP-II proteases is provided in Table 1. Using the sequence alignment of Fig. 2 a model of the JA96 protease can be build using a suitable modelling tool like the Accelrys software Homology , or Modeller (also from Accelrys), or other software like Nest. These programs provide results as a first  
 20 rough model, with some optimization in the Modeller and Nest programs.

The first rough model provides a close structural homology between the model of JA96 protease and the 3D structure of the BCL as there are no overlapping side chains in the model structure. To optimize the structure the protein can *in silico* be soaked in a box of water and subjected to energy minimization and further molecular  
 25 dynamics simulations using e.g. the CHARMM™ software from Accelrys. The *in silico* soaking in water can conveniently be done by adding water in the Insight II program (from Accelrys) with a box size of 75\*75\*75Å<sup>3</sup>. The energy minimization can be done using settings of 300 Steepest descent (SD) and further 600 Conjugated gradients (CJ). The molecular dynamics simulations can conveniently be done using 1.2 ns run  
 30 using the Verlet algorithm at 300K and standard parameters (see CHARMM manual). Other RP-II protease 3D models may be built in an analogous way.

## EXAMPLE 2

### Construction of library of RP-II protease variants

#### Construction and expression of BLC

A *B. subtilis* – *E. coli* shuttle vector, pNM1003, suited to a gene coding for RP-II protease BLC and its mutants was constructed. It is derived from the *B. subtilis* expression vector pSX222 (Described in WO 96/34946) as described in WO 01/16285. To facilitate cloning pNM1008 was constructed introducing a *kpnI* restriction site downstream the *HindIII* site to facilitate the cloning of fragments inside the vector. For transformation in *Bacillus* pNM1008 was restricted with *HindIII* and a 4350 bp DNA fragment was isolated and ligated. The ligation mixture was used to transform competent *B. subtilis* DN1885, selecting for protease activity, as described in WO 01/16285.

#### **Site-directed mutagenesis**

BLC site-directed variants of the invention comprising specific substitutions, insertions or deletions in the molecule were made by traditional cloning of PCR fragments (Sambrook et. al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor) produced by oligonucleotides containing the desired modification. As template pNM1008 was used. In a first PCR using a mutational primer (anti-sense) with a suitable opposite sense primer (e. g., 5'-CTGTGCCCTTTAACCGCACAGC (SEQ ID No. 17)), downstream of the *MluI* site was used. The resulting DNA fragment was used as a sense primer in a second PCR together with a suitable anti-sense primer (e. g. 5'-GCATAAGCTTTTACAGGTACCGGC (SEQ ID No. 18)) upstream from the *KpnI* digestion site. This resulting PCR product was digested with *KpnI* and *MluI* and ligated in pNM1008 digested with the respective enzymes.

The ligation reaction was transformed into *E. coli* by well-known techniques and 5 randomly chosen colonies were sequenced to confirm the designed mutations.

In order to express a BLC variant of the invention, the pNM1008 derived plasmid comprising the variant was digested with *HindIII*, ligated and transformed into a competent *B. subtilis* strain, selecting for protease activity.

## EXAMPLE 3

### **Purification of Enzymes and Variants:**

This procedure relates to purification of 2 liter scale fermentation for the

production of the RP-II proteases of the invention in a *Bacillus* host cell.

Approximately 1.6 liters of fermentation broth are centrifuged at 5000 rpm for 35 minutes in 1 liter beakers. The supernatants are adjusted to pH 7 using 10% acetic acid and filtered through a Seitz Supra S100 filter plate.

5        At room temperature, the filtrate is applied to a 100 ml Bacitracin affinity column equilibrated with 0.01M dimethylglutaric acid, 0.1 M boric acid and 0.002 M calcium chloride adjusted to pH 7 with sodium hydroxide (Buffer A). After washing the column with Buffer A to remove unbound protein, the protease is eluted from the Bacitracin column using Buffer A supplemented with 25% 2-propanol and 1 M sodium chloride.

10       The fractions with protease activity from the Bacitracin purification step are combined and applied to a 750 ml Sephadex G25 column (5 cm dia.) equilibrated with Buffer A.

15       Fractions with proteolytic activity from the Sephadex G25 column are combined and the pH was adjusted to pH 6 with 10% acetic acid and applied to a 150 ml CM Sepharose CL 6B cation exchange column (5 cm dia.) equilibrated with a buffer containing 0.01 M dimethylglutaric acid, 0.1 M boric acid, and 0.002 M calcium chloride adjusted to pH 6 with sodium hydroxide.

The protease is eluted using a linear gradient of 0-0.2 M sodium chloride in 2 liters of the same buffer.

20       Finally, the protease containing fractions from the CM Sepharose column are combined and filtered through a 0.2 $\mu$  filter.

By using the techniques of Example 2 for the construction of variants and fermentation, and the above isolation procedure the following RP-II proteases and variants thereof may be produced and isolated:

25

#### EXAMPLE 4

##### Wash performance of detergent compositions comprising modified enzymes

## AMSA

The enzyme variants of the present application are tested using the Automatic Mechanical Stress Assay (AMSA). With the AMSA test the wash performance of a large quantity of small volume enzyme-detergent solutions can be examined. The AMSA plate has a number of slots for test solutions and a lid firmly squeezing the textile swatch to be washed against all the slot openings. During the washing time, the plate, test solutions, textile and lid are vigorously shaken to bring the test solution in contact with the textile and apply mechanical stress. For further description see WO 02/42740 especially the paragraph "Special method embodiments" at page 23-24.

The assay is conducted under the experimental conditions specified below:

Detergent base	Omo Acao
Detergent dosage	1.5 g/l
Test solution volume	160 micro l
pH	10-10.5 adjusted with NaHCO <sub>3</sub>
Wash time	12 minutes
Temperature	20°C
Water hardness	9°dH
Enzyme concentration in test solution	5 nM, 10 nM and 30 nM
Test material	EMPA 117

After washing the textile pieces are flushed in tap water and air-dried.

The performance of the enzyme variant is measured as the brightness of the colour of the textile samples washed with that specific enzyme variant. Brightness can also be expressed as the intensity of the light reflected from the textile sample when luminated with white light. When the textile is stained the intensity of the reflected light is lower, than that of a clean textile. Therefore the intensity of the reflected light can be used to measure wash performance of an enzyme variant.

Colour measurements are made with a professional flatbed scanner (*PFU DL2400pro*), which is used to capture an image of the washed textile samples. The scans are made with a resolution of 200 dpi and with an output colour dept of 24 bits. In order to get accurate results, the scanner is frequently calibrated with a *Kodak reflective IT8 target*.

To extract a value for the light intensity from the scanned images, a special designed software application is used (*Novozymes Color Vector Analyzer*). The program retrieves the 24 bit pixel values from the image and converts them into values for red, green and blue (RGB). The intensity value (Int) is calculated by adding the RGB values together as vectors and then taking the length of the resulting vector:

$$Int = \sqrt{r^2 + g^2 + b^2}$$

The wash performance (P) of the variants is calculated in accordance with the below formula:

$$P = Int(v) - Int(r)$$

where

Int(v) is the light intensity value of textile surface washed with enzyme variant and Int(r) is the light intensity value of textile surface washed with the reference enzyme, e.g. the parent RP-II protease, BLC or subtilisin 309 (BLSAVI).

The result of the AMSA wash of Hybrid IV is a Performance Score of S (n) in accordance with the definition:

Performance Scores (S) sums the performances (P) of the tested enzyme variants as:

S (2) which indicates that the variant performs better than the reference at all three concentrations (5, 10 and 30 nM) and  
S (1) which indicates that the variant performs better than the reference at one or two concentrations.

## Mini wash assay

The millilitre scale wash performance assay is conducted under the following conditions:

Detergent base	Omo Acao detergent powder
Detergent dose	1.5 g/l
pH	"as is" in the current detergent solution and is not adjusted.
Wash time	14 min.
Temperature	20°C
Water hardness	9°dH, adjusted by adding $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ; $\text{NaHCO}_3$ ( $\text{Ca}^{2+}:\text{Mg}^{2+}:\text{HCO}_3^- = 2:1:6$ ) to milli-Q water.
Enzymes	To be tested/reference
Enzyme conc.	5 nM, 10 nM
Test system	125 ml glass beakers. Textile dipped in test solution. Continuously up and down, 50 times per minute
Textile/volume	1 textile piece (13 x 3 cm) in 50 ml test solution
Test material	EMPA 117 textile swatches

After wash the measurement of remission from the test material is done at 460 nm using a Zeiss MCS 521 VIS spectrophotometer. The measurements are made according to the manufacturer's protocol.

- 5 As shown in Table 1 the textile washed with the RP-II variant at 20°C in Omo Acao has a ???? remission than the textile washed with the parent. This result indicates that this variant has ???? wash performance at low temperature than the parent BLC.

- 10 Table 1. Wash performance results of the RP-II protease variant in Omo Acao for a dosage of 5 nM and 10 nM enzyme.

Enzyme	Remission, 5 nM enzyme	Remission, 10 nM enzyme
Blank (no enzyme)		
BLC		

## Appendix 1

	ATOM	3359	N	SER	B	1	-2.987	12.370	17.565	1.00	7.82	N
	ATOM	3361	CA	SER	B	1	-2.255	12.820	16.353	1.00	7.97	C
5	ATOM	3363	CB	SER	B	1	-3.233	12.933	15.188	1.00	8.69	C
	ATOM	3366	OG	SER	B	1	-3.995	11.748	15.028	1.00	9.01	O
	ATOM	3368	C	SER	B	1	-1.637	14.171	16.602	1.00	8.14	C
	ATOM	3369	O	SER	B	1	-2.098	14.938	17.439	1.00	8.05	O
	ATOM	3372	N	VAL	B	2	-0.592	14.472	15.848	1.00	8.60	N
10	ATOM	3374	CA	VAL	B	2	-0.039	15.812	15.824	1.00	10.11	C
	ATOM	3376	CB	VAL	B	2	1.432	15.811	15.404	1.00	11.81	C
	ATOM	3378	CG1	VAL	B	2	1.949	17.239	15.233	1.00	13.46	C
	ATOM	3382	CG2	VAL	B	2	2.255	15.065	16.421	1.00	14.12	C
	ATOM	3386	C	VAL	B	2	-0.867	16.605	14.830	1.00	10.56	C
15	ATOM	3387	O	VAL	B	2	-0.928	16.250	13.660	1.00	12.81	O
	ATOM	3388	N	ILE	B	3	-1.524	17.640	15.331	1.00	9.91	N
	ATOM	3390	CA	ILE	B	3	-2.409	18.487	14.537	1.00	10.49	C
	ATOM	3392	CB	ILE	B	3	-3.747	18.700	15.279	1.00	10.68	C
	ATOM	3394	CG1	ILE	B	3	-4.452	17.348	15.457	1.00	10.36	C
20	ATOM	3397	CD1	ILE	B	3	-5.671	17.398	16.350	1.00	11.17	C
	ATOM	3401	CG2	ILE	B	3	-4.638	19.704	14.531	1.00	13.34	C
	ATOM	3405	C	ILE	B	3	-1.683	19.796	14.299	1.00	10.96	C
	ATOM	3406	O	ILE	B	3	-1.332	20.502	15.234	1.00	10.91	O
	ATOM	3407	N	GLY	B	4	-1.433	20.141	13.043	1.00	12.22	N
25	ATOM	3409	CA	GLY	B	4	-0.702	21.359	12.748	1.00	12.69	C
	ATOM	3412	C	GLY	B	4	0.685	21.285	13.344	1.00	12.61	C
	ATOM	3413	O	GLY	B	4	1.324	20.239	13.303	1.00	13.40	O
	ATOM	3414	N	SER	B	5	1.162	22.383	13.913	1.00	11.93	N
	ATOM	3416	CA	SER	B	5	2.466	22.358	14.557	1.00	11.64	C
30	ATOM	3418	CB	SER	B	5	2.900	23.757	14.975	1.00	11.92	C
	ATOM	3421	OG	SER	B	5	2.011	24.329	15.906	1.00	13.28	O
	ATOM	3423	C	SER	B	5	2.438	21.451	15.770	1.00	11.22	C
	ATOM	3424	O	SER	B	5	1.437	21.366	16.462	1.00	11.19	O
	ATOM	3425	N	ASP	B	6	3.551	20.779	16.028	1.00	10.41	N
35	ATOM	3427	CA	ASP	B	6	3.704	19.951	17.230	1.00	10.02	C
	ATOM	3429	CB	ASP	B	6	4.700	18.839	16.981	1.00	10.75	C
	ATOM	3432	CG	ASP	B	6	4.838	17.886	18.144	1.00	10.38	C
	ATOM	3433	OD1	ASP	B	6	4.132	18.013	19.178	1.00	10.80	O
	ATOM	3434	OD2	ASP	B	6	5.685	16.961	18.055	1.00	11.46	O
40	ATOM	3435	C	ASP	B	6	4.185	20.807	18.373	1.00	9.61	C
	ATOM	3436	O	ASP	B	6	5.353	21.229	18.410	1.00	11.09	O
	ATOM	3437	N	ASP	B	7	3.290	21.057	19.312	1.00	8.85	N
	ATOM	3439	CA	ASP	B	7	3.582	21.969	20.387	1.00	8.21	C
	ATOM	3441	CB	ASP	B	7	2.453	23.010	20.550	1.00	9.26	C
45	ATOM	3444	CG	ASP	B	7	2.334	23.975	19.386	1.00	10.17	C
	ATOM	3445	OD1	ASP	B	7	3.147	23.902	18.444	1.00	11.15	O
	ATOM	3446	OD2	ASP	B	7	1.377	24.778	19.332	1.00	10.99	O
	ATOM	3447	C	ASP	B	7	3.856	21.237	21.712	1.00	8.24	C
	ATOM	3448	O	ASP	B	7	3.978	21.870	22.753	1.00	8.50	O
50	ATOM	3449	N	ARG	B	8	4.016	19.918	21.677	1.00	7.90	N
	ATOM	3451	CA	ARG	B	8	4.429	19.187	22.872	1.00	7.81	C
	ATOM	3453	CB	ARG	B	8	4.444	17.681	22.634	1.00	7.75	C
	ATOM	3456	CG	ARG	B	8	3.068	17.077	22.470	1.00	7.65	C
	ATOM	3459	CD	ARG	B	8	3.090	15.631	22.015	1.00	7.89	C
55	ATOM	3462	NE	ARG	B	8	3.673	15.554	20.679	1.00	8.24	N
	ATOM	3464	CZ	ARG	B	8	4.023	14.422	20.073	1.00	8.49	C
	ATOM	3465	NH1	ARG	B	8	3.781	13.244	20.628	1.00	8.61	N
	ATOM	3468	NH2	ARG	B	8	4.622	14.472	18.909	1.00	9.63	N
	ATOM	3471	C	ARG	B	8	5.812	19.628	23.321	1.00	8.24	C
60	ATOM	3472	O	ARG	B	8	6.684	19.907	22.505	1.00	9.34	O
	ATOM	3473	N	THR	B	9	6.007	19.640	24.632	1.00	8.26	N
	ATOM	3475	CA	THR	B	9	7.315	19.897	25.226	1.00	8.75	C
	ATOM	3477	CB	THR	B	9	7.368	21.243	25.939	1.00	9.87	C
	ATOM	3479	OG1	THR	B	9	6.296	21.350	26.880	1.00	10.91	O

	ATOM	3481	CG2	THR	B	9	7.191	22.375	24.936	1.00	11.78	C
	ATOM	3485	C	THR	B	9	7.660	18.787	26.199	1.00	8.34	C
	ATOM	3486	O	THR	B	9	6.793	18.176	26.835	1.00	8.22	O
5	ATOM	3487	N	ARG	B	10	8.954	18.535	26.340	1.00	8.65	N
	ATOM	3489	CA	ARG	B	10	9.413	17.459	27.194	1.00	8.98	C
	ATOM	3491	CB	ARG	B	10	10.873	17.096	26.927	1.00	10.45	C
	ATOM	3494	CG	ARG	B	10	11.309	15.787	27.587	1.00	11.25	C
	ATOM	3497	CD	ARG	B	10	12.701	15.396	27.212	1.00	12.23	C
10	ATOM	3500	NE	ARG	B	10	13.213	14.299	28.025	1.00	12.62	N
	ATOM	3502	CZ	ARG	B	10	14.465	13.868	27.967	1.00	14.40	C
	ATOM	3503	NH1	ARG	B	10	15.328	14.413	27.114	1.00	16.93	N
	ATOM	3506	NH2	ARG	B	10	14.855	12.884	28.743	1.00	14.13	N
	ATOM	3509	C	ARG	B	10	9.237	17.885	28.642	1.00	8.65	C
15	ATOM	3510	O	ARG	B	10	9.534	19.027	29.025	1.00	9.59	O
	ATOM	3511	N	VAL	B	11	8.771	16.952	29.453	1.00	8.69	N
	ATOM	3513	CA	VAL	B	11	8.751	17.118	30.893	1.00	9.52	C
	ATOM	3515	CB	VAL	B	11	7.810	16.080	31.532	1.00	9.21	C
	ATOM	3517	CG1	VAL	B	11	7.862	16.145	33.047	1.00	10.41	C
20	ATOM	3521	CG2	VAL	B	11	6.381	16.257	31.015	1.00	9.54	C
	ATOM	3525	C	VAL	B	11	10.207	16.954	31.390	1.00	10.62	C
	ATOM	3526	O	VAL	B	11	10.777	15.869	31.301	1.00	12.34	O
	ATOM	3527	N	THR	B	12	10.795	18.048	31.884	1.00	12.38	N
	ATOM	3529	CA	THR	B	12	12.217	18.113	32.253	1.00	13.55	C
25	ATOM	3531	CB	THR	B	12	12.790	19.543	32.093	1.00	14.37	C
	ATOM	3533	OG1	THR	B	12	12.035	20.449	32.902	1.00	17.60	O
	ATOM	3535	CG2	THR	B	12	12.611	20.030	30.671	1.00	16.03	C
	ATOM	3539	C	THR	B	12	12.507	17.657	33.666	1.00	13.34	C
	ATOM	3540	O	THR	B	12	13.669	17.515	34.032	1.00	14.60	O
30	ATOM	3541	N	ASN	B	13	11.472	17.465	34.469	1.00	12.04	N
	ATOM	3543	CA	ASN	B	13	11.646	16.901	35.800	1.00	11.12	C
	ATOM	3545	CB	ASN	B	13	11.713	17.962	36.894	1.00	11.74	C
	ATOM	3548	CG	ASN	B	13	11.935	17.344	38.252	1.00	12.29	C
	ATOM	3549	OD1	ASN	B	13	12.166	16.141	38.356	1.00	12.18	O
35	ATOM	3550	ND2	ASN	B	13	11.868	18.153	39.302	1.00	15.45	N
	ATOM	3553	C	ASN	B	13	10.502	15.940	36.074	1.00	10.21	C
	ATOM	3554	O	ASN	B	13	9.450	16.321	36.578	1.00	10.60	O
	ATOM	3555	N	THR	B	14	10.714	14.678	35.743	1.00	9.43	N
	ATOM	3557	CA	THR	B	14	9.671	13.680	35.934	1.00	9.11	C
40	ATOM	3559	CB	THR	B	14	9.887	12.455	35.046	1.00	9.24	C
	ATOM	3561	OG1	THR	B	14	11.122	11.827	35.409	1.00	9.63	O
	ATOM	3563	CG2	THR	B	14	9.958	12.808	33.561	1.00	10.29	C
	ATOM	3567	C	THR	B	14	9.556	13.227	37.385	1.00	9.62	C
	ATOM	3568	O	THR	B	14	8.730	12.361	37.672	1.00	10.68	O
45	ATOM	3569	N	THR	B	15	10.357	13.804	38.295	1.00	10.09	N
	ATOM	3571	CA	THR	B	15	10.147	13.593	39.725	1.00	10.57	C
	ATOM	3573	CB	THR	B	15	11.456	13.495	40.553	1.00	11.89	C
	ATOM	3575	OG1	THR	B	15	12.124	14.763	40.616	1.00	12.96	O
	ATOM	3577	CG2	THR	B	15	12.432	12.491	39.954	1.00	12.96	C
50	ATOM	3581	C	THR	B	15	9.244	14.638	40.367	1.00	10.41	C
	ATOM	3582	O	THR	B	15	8.911	14.514	41.540	1.00	12.03	O
	ATOM	3583	N	ALA	B	16	8.832	15.656	39.622	1.00	10.32	N
	ATOM	3585	CA	ALA	B	16	7.900	16.643	40.148	1.00	10.73	C
	ATOM	3587	CB	ALA	B	16	7.927	17.897	39.301	1.00	11.48	C
55	ATOM	3591	C	ALA	B	16	6.488	16.060	40.161	1.00	10.05	C
	ATOM	3592	O	ALA	B	16	6.059	15.433	39.198	1.00	9.80	O
	ATOM	3593	N	TYR	B	17	5.755	16.284	41.237	1.00	10.35	N
	ATOM	3595	CA	TYR	B	17	4.338	15.962	41.260	1.00	10.36	C
	ATOM	3597	CB	TYR	B	17	3.838	16.018	42.706	1.00	10.90	C
60	ATOM	3600	CG	TYR	B	17	2.379	15.675	42.858	1.00	10.77	C
	ATOM	3601	CD1	TYR	B	17	1.436	16.674	42.985	1.00	11.41	C
	ATOM	3603	CE1	TYR	B	17	0.086	16.386	43.118	1.00	11.35	C
	ATOM	3605	CZ	TYR	B	17	-0.338	15.081	43.139	1.00	11.51	C
	ATOM	3606	OH	TYR	B	17	-1.690	14.831	43.268	1.00	13.22	O
	ATOM	3608	CE2	TYR	B	17	0.579	14.051	42.988	1.00	11.13	C

	ATOM	3610	CD2	TYR	B	17	1.940	14.358	42.861	1.00	11.24	C
	ATOM	3612	C	TYR	B	17	3.588	16.946	40.363	1.00	10.06	C
	ATOM	3613	O	TYR	B	17	3.857	18.150	40.452	1.00	11.57	O
5	ATOM	3614	N	PRO	B	18	2.609	16.510	39.557	1.00	10.05	N
	ATOM	3615	CA	PRO	B	18	2.080	15.145	39.436	1.00	9.55	C
	ATOM	3617	CB	PRO	B	18	0.606	15.412	39.151	1.00	10.69	C
	ATOM	3620	CG	PRO	B	18	0.646	16.604	38.275	1.00	11.31	C
	ATOM	3623	CD	PRO	B	18	1.772	17.460	38.810	1.00	10.99	C
10	ATOM	3626	C	PRO	B	18	2.667	14.326	38.287	1.00	8.62	C
	ATOM	3627	O	PRO	B	18	2.189	13.217	38.035	1.00	8.43	O
	ATOM	3628	N	TYR	B	19	3.695	14.844	37.616	1.00	8.36	N
	ATOM	3630	CA	TYR	B	19	4.343	14.126	36.531	1.00	8.21	C
	ATOM	3632	CB	TYR	B	19	5.389	15.034	35.875	1.00	8.56	C
15	ATOM	3635	CG	TYR	B	19	4.722	16.277	35.304	1.00	8.70	C
	ATOM	3636	CD1	TYR	B	19	4.072	16.231	34.070	1.00	8.24	C
	ATOM	3638	CE1	TYR	B	19	3.424	17.343	33.553	1.00	9.10	C
	ATOM	3640	CZ	TYR	B	19	3.374	18.496	34.286	1.00	9.96	C
	ATOM	3641	OH	TYR	B	19	2.725	19.608	33.802	1.00	11.01	O
20	ATOM	3643	CE2	TYR	B	19	3.987	18.565	35.519	1.00	10.79	C
	ATOM	3645	CD2	TYR	B	19	4.660	17.462	36.020	1.00	10.02	C
	ATOM	3647	C	TYR	B	19	4.951	12.801	36.969	1.00	7.80	C
	ATOM	3648	O	TYR	B	19	4.984	11.860	36.180	1.00	8.04	O
	ATOM	3649	N	ARG	B	20	5.385	12.701	38.224	1.00	7.62	N
25	ATOM	3651	CA	ARG	B	20	5.919	11.452	38.741	1.00	7.92	C
	ATOM	3653	CB	ARG	B	20	6.659	11.679	40.056	1.00	8.70	C
	ATOM	3656	CG	ARG	B	20	5.865	12.292	41.176	1.00	9.58	C
	ATOM	3659	CD	ARG	B	20	6.640	12.228	42.469	1.00	10.61	C
	ATOM	3662	NE	ARG	B	20	5.937	12.768	43.620	1.00	12.27	N
	ATOM	3664	CZ	ARG	B	20	6.343	13.830	44.332	1.00	14.55	C
30	ATOM	3665	NH1	ARG	B	20	7.433	14.528	44.011	1.00	15.43	N
	ATOM	3668	NH2	ARG	B	20	5.641	14.205	45.395	1.00	15.98	N
	ATOM	3671	C	ARG	B	20	4.833	10.398	38.938	1.00	7.88	C
	ATOM	3672	O	ARG	B	20	5.142	9.210	39.062	1.00	8.74	O
35	ATOM	3673	N	ALA	B	21	3.573	10.834	38.989	1.00	7.67	N
	ATOM	3675	CA	ALA	B	21	2.436	9.931	39.101	1.00	7.77	C
	ATOM	3677	CB	ALA	B	21	1.355	10.545	40.004	1.00	8.33	C
	ATOM	3681	C	ALA	B	21	1.860	9.554	37.740	1.00	7.49	C
	ATOM	3682	O	ALA	B	21	0.883	8.813	37.670	1.00	8.24	O
40	ATOM	3683	N	ILE	B	22	2.451	10.077	36.668	1.00	7.07	N
	ATOM	3685	CA	ILE	B	22	2.180	9.629	35.315	1.00	7.15	C
	ATOM	3687	CB	ILE	B	22	2.239	10.805	34.320	1.00	7.19	C
	ATOM	3689	CG1	ILE	B	22	1.204	11.861	34.727	1.00	7.74	C
	ATOM	3692	CD1	ILE	B	22	1.150	13.060	33.823	1.00	7.78	C
45	ATOM	3696	CG2	ILE	B	22	2.012	10.301	32.895	1.00	7.55	C
	ATOM	3700	C	ILE	B	22	3.192	8.540	35.014	1.00	7.08	C
	ATOM	3701	O	ILE	B	22	4.376	8.686	35.297	1.00	8.15	O
	ATOM	3702	N	VAL	B	23	2.708	7.426	34.477	1.00	7.33	N
	ATOM	3704	CA	VAL	B	23	3.505	6.221	34.384	1.00	7.49	C
50	ATOM	3706	CB	VAL	B	23	2.933	5.092	35.284	1.00	7.65	C
	ATOM	3708	CG1	VAL	B	23	2.619	5.599	36.672	1.00	8.69	C
	ATOM	3712	CG2	VAL	B	23	1.690	4.436	34.682	1.00	8.21	C
	ATOM	3716	C	VAL	B	23	3.625	5.760	32.939	1.00	6.99	C
	ATOM	3717	O	VAL	B	23	2.710	5.912	32.130	1.00	7.44	O
	ATOM	3718	N	HIS	B	24	4.788	5.194	32.623	1.00	7.09	N
55	ATOM	3720	CA	HIS	B	24	5.005	4.494	31.375	1.00	7.24	C
	ATOM	3722	CB	HIS	B	24	6.484	4.596	30.984	1.00	7.56	C
	ATOM	3725	CG	HIS	B	24	6.810	3.808	29.779	1.00	8.11	C
	ATOM	3726	ND1	HIS	B	24	7.112	2.467	29.831	1.00	9.52	N
	ATOM	3728	CE1	HIS	B	24	7.263	2.022	28.599	1.00	10.58	C
60	ATOM	3730	NE2	HIS	B	24	7.090	3.026	27.757	1.00	11.37	N
	ATOM	3732	CD2	HIS	B	24	6.804	4.156	28.474	1.00	10.43	C
	ATOM	3734	C	HIS	B	24	4.599	3.027	31.568	1.00	7.57	C
	ATOM	3735	O	HIS	B	24	4.949	2.409	32.577	1.00	8.17	O
	ATOM	3736	N	ILE	B	25	3.848	2.485	30.615	1.00	7.37	N

	ATOM	3738	CA	ILE	B	25	3.381	1.108	30.652	1.00	7.87	C
	ATOM	3740	CB	ILE	B	25	1.842	1.058	30.651	1.00	8.18	C
	ATOM	3742	CG1	ILE	B	25	1.257	1.843	31.824	1.00	9.00	C
5	ATOM	3745	CD1	ILE	B	25	-0.242	2.093	31.705	1.00	8.99	C
	ATOM	3749	CG2	ILE	B	25	1.356	-0.398	30.666	1.00	9.66	C
	ATOM	3753	C	ILE	B	25	3.899	0.364	29.441	1.00	8.15	C
	ATOM	3754	O	ILE	B	25	3.755	0.843	28.315	1.00	8.94	O
	ATOM	3755	N	SER	B	26	4.486	-0.806	29.669	1.00	8.77	N
	ATOM	3757	CA	SER	B	26	4.773	-1.727	28.581	1.00	9.89	C
10	ATOM	3759	CB	BSER	B	26	6.238	-1.804	28.196	0.35	10.66	C
	ATOM	3760	CB	ASER	B	26	6.305	-1.864	28.514	0.65	11.47	C
	ATOM	3765	OG	BSER	B	26	6.986	-2.328	29.246	0.35	11.77	O
	ATOM	3766	OG	ASER	B	26	6.755	-2.916	27.701	0.65	12.82	O
	ATOM	3769	C	SER	B	26	4.177	-3.089	28.889	1.00	9.15	C
15	ATOM	3770	O	SER	B	26	4.245	-3.579	30.017	1.00	9.90	O
	ATOM	3771	N	SER	B	27	3.579	-3.695	27.878	1.00	8.91	N
	ATOM	3773	CA	SER	B	27	3.049	-5.042	27.993	1.00	9.24	C
	ATOM	3775	CB	SER	B	27	1.609	-5.020	28.523	1.00	9.75	C
	ATOM	3778	OG	SER	B	27	0.701	-4.659	27.498	1.00	10.07	O
20	ATOM	3780	C	SER	B	27	3.045	-5.686	26.626	1.00	9.09	C
	ATOM	3781	O	SER	B	27	3.418	-5.071	25.633	1.00	9.64	O
	ATOM	3782	N	SER	B	28	2.555	-6.913	26.573	1.00	9.24	N
	ATOM	3784	CA	SER	B	28	2.448	-7.620	25.319	1.00	9.63	C
	ATOM	3786	CB	SER	B	28	1.950	-9.034	25.569	1.00	10.05	C
25	ATOM	3789	OG	SER	B	28	0.663	-9.022	26.149	1.00	11.00	O
	ATOM	3791	C	SER	B	28	1.551	-6.906	24.309	1.00	9.09	C
	ATOM	3792	O	SER	B	28	1.683	-7.141	23.109	1.00	10.26	O
	ATOM	3793	N	ILE	B	29	0.612	-6.081	24.765	1.00	9.01	N
	ATOM	3795	CA	ILE	B	29	-0.230	-5.322	23.829	1.00	9.45	C
30	ATOM	3797	CB	ILE	B	29	-1.528	-4.860	24.527	1.00	9.84	C
	ATOM	3799	CG1	ILE	B	29	-2.467	-6.054	24.687	1.00	10.68	C
	ATOM	3802	CD1	ILE	B	29	-3.749	-5.729	25.407	1.00	11.23	C
	ATOM	3806	CG2	ILE	B	29	-2.209	-3.738	23.755	1.00	10.93	C
	ATOM	3810	C	ILE	B	29	0.520	-4.165	23.182	1.00	9.75	C
35	ATOM	3811	O	ILE	B	29	0.298	-3.856	22.009	1.00	10.61	O
	ATOM	3812	N	GLY	B	30	1.392	-3.519	23.936	1.00	9.50	N
	ATOM	3814	CA	GLY	B	30	2.104	-2.366	23.439	1.00	10.18	C
	ATOM	3817	C	GLY	B	30	2.498	-1.451	24.564	1.00	8.93	C
	ATOM	3818	O	GLY	B	30	2.432	-1.827	25.728	1.00	10.65	O
40	ATOM	3819	N	SER	B	31	2.926	-0.258	24.195	1.00	9.21	N
	ATOM	3821	CA	SER	B	31	3.322	0.746	25.151	1.00	9.76	C
	ATOM	3823	CB	BSER	B	31	4.627	1.413	24.672	0.35	10.79	C
	ATOM	3824	CB	ASER	B	31	4.636	1.385	24.762	0.65	11.07	C
	ATOM	3829	OG	BSER	B	31	5.007	2.545	25.442	0.35	12.74	O
45	ATOM	3830	OG	ASER	B	31	5.642	0.393	24.813	0.65	12.96	O
	ATOM	3833	C	SER	B	31	2.236	1.796	25.263	1.00	8.79	C
	ATOM	3834	O	SER	B	31	1.624	2.194	24.261	1.00	10.03	O
	ATOM	3835	N	CYS	B	32	2.006	2.249	26.481	1.00	8.21	N
	ATOM	3837	CA	CYS	B	32	0.981	3.237	26.755	1.00	8.25	C
50	ATOM	3839	CB	BCYS	B	32	-0.398	2.638	26.853	0.35	9.91	C
	ATOM	3840	CB	ACYS	B	32	-0.338	2.497	27.106	0.65	8.79	C
	ATOM	3845	SG	BCYS	B	32	-0.604	1.615	28.261	0.35	14.50	S
	ATOM	3846	SG	ACYS	B	32	-1.274	1.895	25.659	0.65	7.95	S
	ATOM	3847	C	CYS	B	32	1.399	4.076	27.956	1.00	7.16	C
55	ATOM	3848	O	CYS	B	32	2.526	3.975	28.467	1.00	8.13	O
	ATOM	3849	N	THR	B	33	0.491	4.947	28.359	1.00	6.54	N
	ATOM	3851	CA	THR	B	33	0.647	5.783	29.522	1.00	6.41	C
	ATOM	3853	CB	THR	B	33	0.515	7.251	29.080	1.00	6.34	C
	ATOM	3855	OG1	THR	B	33	1.515	7.524	28.079	1.00	6.92	O
60	ATOM	3857	CG2	THR	B	33	0.761	8.237	30.220	1.00	6.68	C
	ATOM	3861	C	THR	B	33	-0.451	5.417	30.520	1.00	6.49	C
	ATOM	3862	O	THR	B	33	-1.496	4.893	30.137	1.00	6.80	O
	ATOM	3863	N	GLY	B	34	-0.228	5.715	31.793	1.00	6.76	N
	ATOM	3865	CA	GLY	B	34	-1.290	5.682	32.779	1.00	6.72	C

	ATOM	3868	C	GLY	B	34	-1.039	6.736	33.827	1.00	6.52	C
	ATOM	3869	O	GLY	B	34	-0.075	7.493	33.760	1.00	6.78	O
	ATOM	3870	N	TRP	B	35	-1.887	6.753	34.838	1.00	6.86	N
5	ATOM	3872	CA	TRP	B	35	-1.766	7.724	35.904	1.00	7.26	C
	ATOM	3874	CB	TRP	B	35	-2.492	9.043	35.563	1.00	7.82	C
	ATOM	3877	CG	TRP	B	35	-3.831	8.901	34.906	1.00	8.11	C
	ATOM	3878	CD1	TRP	B	35	-4.066	8.555	33.608	1.00	8.12	C
	ATOM	3880	NE1	TRP	B	35	-5.414	8.580	33.339	1.00	8.93	N
10	ATOM	3882	CE2	TRP	B	35	-6.079	8.965	34.473	1.00	8.81	C
	ATOM	3883	CD2	TRP	B	35	-5.111	9.181	35.475	1.00	7.96	C
	ATOM	3884	CE3	TRP	B	35	-5.542	9.590	36.735	1.00	8.75	C
	ATOM	3886	CZ3	TRP	B	35	-6.887	9.760	36.966	1.00	9.89	C
	ATOM	3888	CH2	TRP	B	35	-7.814	9.526	35.963	1.00	10.09	C
	ATOM	3890	CZ2	TRP	B	35	-7.432	9.140	34.705	1.00	10.05	C
15	ATOM	3892	C	TRP	B	35	-2.265	7.119	37.203	1.00	7.17	C
	ATOM	3893	O	TRP	B	35	-3.305	6.444	37.247	1.00	7.48	O
	ATOM	3894	N	MET	B	36	-1.514	7.324	38.276	1.00	7.22	N
	ATOM	3896	CA	MET	B	36	-1.884	6.750	39.562	1.00	7.60	C
20	ATOM	3898	CB	MET	B	36	-0.790	6.983	40.601	1.00	8.12	C
	ATOM	3901	CG	MET	B	36	0.593	6.429	40.265	1.00	8.68	C
	ATOM	3904	SD	MET	B	36	0.683	4.684	39.895	1.00	9.14	S
	ATOM	3905	CE	MET	B	36	0.098	4.015	41.440	1.00	9.93	C
	ATOM	3909	C	MET	B	36	-3.173	7.378	40.084	1.00	7.70	C
25	ATOM	3910	O	MET	B	36	-3.339	8.603	40.029	1.00	8.47	O
	ATOM	3911	N	ILE	B	37	-4.055	6.534	40.632	1.00	7.60	N
	ATOM	3913	CA	ILE	B	37	-5.248	6.992	41.337	1.00	8.62	C
	ATOM	3915	CB	ILE	B	37	-6.553	6.614	40.591	1.00	8.72	C
	ATOM	3917	CG1	ILE	B	37	-6.723	5.099	40.438	1.00	9.33	C
	ATOM	3920	CD1	ILE	B	37	-8.120	4.724	39.928	1.00	9.73	C
30	ATOM	3924	CG2	ILE	B	37	-6.607	7.330	39.261	1.00	9.21	C
	ATOM	3928	C	ILE	B	37	-5.294	6.519	42.789	1.00	8.85	C
	ATOM	3929	O	ILE	B	37	-6.214	6.872	43.524	1.00	10.47	O
	ATOM	3930	N	GLY	B	38	-4.311	5.739	43.210	1.00	9.34	N
35	ATOM	3932	CA	GLY	B	38	-4.205	5.289	44.585	1.00	9.66	C
	ATOM	3935	C	GLY	B	38	-2.837	4.675	44.794	1.00	9.97	C
	ATOM	3936	O	GLY	B	38	-1.986	4.723	43.900	1.00	10.35	O
	ATOM	3937	N	PRO	B	39	-2.597	4.131	45.975	1.00	9.86	N
	ATOM	3938	CA	PRO	B	39	-1.304	3.498	46.274	1.00	10.14	C
40	ATOM	3940	CB	PRO	B	39	-1.552	2.839	47.634	1.00	10.75	C
	ATOM	3943	CG	PRO	B	39	-2.545	3.766	48.271	1.00	11.80	C
	ATOM	3946	CD	PRO	B	39	-3.486	4.139	47.149	1.00	10.25	C
	ATOM	3949	C	PRO	B	39	-0.830	2.487	45.238	1.00	9.69	C
	ATOM	3950	O	PRO	B	39	0.366	2.411	44.978	1.00	10.04	O
45	ATOM	3951	N	LYS	B	40	-1.734	1.687	44.702	1.00	9.60	N
	ATOM	3953	CA	LYS	B	40	-1.328	0.634	43.791	1.00	9.71	C
	ATOM	3955	CB	LYS	B	40	-1.113	-0.678	44.529	1.00	11.09	C
	ATOM	3958	CG	LYS	B	40	-2.335	-1.186	45.229	1.00	11.94	C
	ATOM	3961	CD	LYS	B	40	-2.132	-2.615	45.726	1.00	13.45	C
	ATOM	3964	CE	LYS	B	40	-0.996	-2.749	46.704	1.00	14.20	C
50	ATOM	3967	NZ	LYS	B	40	-0.976	-4.121	47.344	1.00	15.10	N
	ATOM	3971	C	LYS	B	40	-2.284	0.467	42.617	1.00	8.70	C
	ATOM	3972	O	LYS	B	40	-2.366	-0.617	42.060	1.00	9.87	O
	ATOM	3973	N	THR	B	41	-2.985	1.532	42.227	1.00	8.11	N
55	ATOM	3975	CA	THR	B	41	-3.939	1.455	41.125	1.00	8.14	C
	ATOM	3977	CB	THR	B	41	-5.375	1.586	41.663	1.00	8.25	C
	ATOM	3979	OG1	THR	B	41	-5.572	0.652	42.741	1.00	9.37	O
	ATOM	3981	CG2	THR	B	41	-6.399	1.262	40.576	1.00	9.16	C
	ATOM	3985	C	THR	B	41	-3.641	2.556	40.130	1.00	7.63	C
60	ATOM	3986	O	THR	B	41	-3.476	3.711	40.515	1.00	8.27	O
	ATOM	3987	N	VAL	B	42	-3.590	2.160	38.861	1.00	7.48	N
	ATOM	3989	CA	VAL	B	42	-3.271	3.007	37.732	1.00	7.56	C
	ATOM	3991	CB	VAL	B	42	-2.122	2.378	36.911	1.00	7.80	C
	ATOM	3993	CG1	VAL	B	42	-1.745	3.260	35.729	1.00	8.94	C
	ATOM	3997	CG2	VAL	B	42	-0.914	2.085	37.763	1.00	9.62	C

	ATOM	4001	C	VAL	B	42	-4.491	3.072	36.818	1.00	7.34	C
	ATOM	4002	O	VAL	B	42	-5.024	2.044	36.433	1.00	9.14	O
	ATOM	4003	N	ALA	B	43	-4.918	4.274	36.432	1.00	7.37	N
5	ATOM	4005	CA	ALA	B	43	-5.911	4.442	35.377	1.00	7.20	C
	ATOM	4007	CB	ALA	B	43	-6.711	5.713	35.603	1.00	7.51	C
	ATOM	4011	C	ALA	B	43	-5.214	4.503	34.017	1.00	7.00	C
	ATOM	4012	O	ALA	B	43	-4.129	5.081	33.886	1.00	7.26	O
	ATOM	4013	N	THR	B	44	-5.836	3.904	33.019	1.00	6.97	N
	ATOM	4015	CA	THR	B	44	-5.286	3.897	31.670	1.00	7.04	C
10	ATOM	4017	CB	THR	B	44	-4.160	2.834	31.570	1.00	7.41	C
	ATOM	4019	OG1	THR	B	44	-3.485	2.938	30.303	1.00	7.54	O
	ATOM	4021	CG2	THR	B	44	-4.692	1.413	31.698	1.00	7.72	C
	ATOM	4025	C	THR	B	44	-6.413	3.683	30.656	1.00	6.99	C
	ATOM	4026	O	THR	B	44	-7.596	3.731	30.998	1.00	7.52	O
15	ATOM	4027	N	ALA	B	45	-6.048	3.485	29.395	1.00	7.00	N
	ATOM	4029	CA	ALA	B	45	-7.003	3.149	28.349	1.00	7.12	C
	ATOM	4031	CB	ALA	B	45	-6.479	3.579	26.979	1.00	7.53	C
	ATOM	4035	C	ALA	B	45	-7.281	1.644	28.351	1.00	7.28	C
	ATOM	4036	O	ALA	B	45	-6.370	0.833	28.543	1.00	8.36	O
20	ATOM	4037	N	GLY	B	46	-8.529	1.256	28.120	1.00	7.41	N
	ATOM	4039	CA	GLY	B	46	-8.874	-0.156	28.014	1.00	7.78	C
	ATOM	4042	C	GLY	B	46	-8.106	-0.884	26.933	1.00	7.87	C
	ATOM	4043	O	GLY	B	46	-7.669	-2.017	27.135	1.00	8.48	O
	ATOM	4044	N	HIS	B	47	-7.940	-0.234	25.783	1.00	7.88	N
25	ATOM	4046	CA	HIS	B	47	-7.288	-0.893	24.672	1.00	8.40	C
	ATOM	4048	CB	HIS	B	47	-7.524	-0.133	23.362	1.00	8.56	C
	ATOM	4051	CG	HIS	B	47	-6.718	1.122	23.182	1.00	7.89	C
	ATOM	4052	ND1	HIS	B	47	-7.280	2.381	23.233	1.00	8.37	N
	ATOM	4054	CE1	HIS	B	47	-6.356	3.284	22.954	1.00	8.17	C
30	ATOM	4056	NE2	HIS	B	47	-5.209	2.668	22.753	1.00	8.05	N
	ATOM	4058	CD2	HIS	B	47	-5.409	1.313	22.884	1.00	7.79	C
	ATOM	4060	C	HIS	B	47	-5.808	-1.162	24.909	1.00	8.34	C
	ATOM	4061	O	HIS	B	47	-5.198	-1.909	24.160	1.00	9.86	O
	ATOM	4062	N	CYS	B	48	-5.235	-0.537	25.933	1.00	7.91	N
35	ATOM	4064	CA	CYS	B	48	-3.850	-0.803	26.311	1.00	8.43	C
	ATOM	4066	CB	CYS	B	48	-3.317	0.340	27.164	1.00	9.43	C
	ATOM	4069	SG	CYS	B	48	-3.197	1.908	26.286	1.00	11.14	S
	ATOM	4070	C	CYS	B	48	-3.671	-2.102	27.099	1.00	8.41	C
	ATOM	4071	O	CYS	B	48	-2.553	-2.599	27.197	1.00	9.30	O
40	ATOM	4072	N	ILE	B	49	-4.758	-2.622	27.679	1.00	8.25	N
	ATOM	4074	CA	ILE	B	49	-4.680	-3.771	28.589	1.00	8.11	C
	ATOM	4076	CB	ILE	B	49	-4.931	-3.327	30.049	1.00	8.38	C
	ATOM	4078	CG1	ILE	B	49	-6.349	-2.791	30.254	1.00	8.89	C
	ATOM	4081	CD1	ILE	B	49	-6.631	-2.365	31.696	1.00	9.33	C
45	ATOM	4085	CG2	ILE	B	49	-3.871	-2.314	30.454	1.00	9.04	C
	ATOM	4089	C	ILE	B	49	-5.574	-4.945	28.224	1.00	8.36	C
	ATOM	4090	O	ILE	B	49	-5.385	-6.015	28.774	1.00	8.42	O
	ATOM	4091	N	TYR	B	50	-6.527	-4.765	27.313	1.00	8.78	N
	ATOM	4093	CA	TYR	B	50	-7.397	-5.847	26.876	1.00	9.04	C
50	ATOM	4095	CB	TYR	B	50	-8.752	-5.812	27.602	1.00	9.41	C
	ATOM	4098	CG	TYR	B	50	-9.689	-6.905	27.142	1.00	10.04	C
	ATOM	4099	CD1	TYR	B	50	-10.686	-6.650	26.211	1.00	10.86	C
	ATOM	4101	CE1	TYR	B	50	-11.534	-7.668	25.770	1.00	11.77	C
	ATOM	4103	CZ	TYR	B	50	-11.372	-8.951	26.279	1.00	11.98	C
55	ATOM	4104	OH	TYR	B	50	-12.188	-9.993	25.878	1.00	14.06	O
	ATOM	4106	CE2	TYR	B	50	-10.394	-9.208	27.210	1.00	11.89	C
	ATOM	4108	CD2	TYR	B	50	-9.549	-8.200	27.615	1.00	10.91	C
	ATOM	4110	C	TYR	B	50	-7.585	-5.731	25.363	1.00	9.64	C
	ATOM	4111	O	TYR	B	50	-8.007	-4.678	24.858	1.00	10.03	O
60	ATOM	4112	N	ASP	B	51	-7.221	-6.802	24.663	1.00	10.47	N
	ATOM	4114	CA	ASP	B	51	-7.291	-6.906	23.220	1.00	12.23	C
	ATOM	4116	CB	BASP	B	51	-6.107	-7.742	22.729	0.35	12.66	C
	ATOM	4117	CB	AASP	B	51	-6.122	-7.695	22.640	0.65	13.16	C
	ATOM	4122	CG	BASP	B	51	-6.080	-7.888	21.234	0.35	13.82	C

	ATOM	4123	CG	AASP	B	51	-6.149	-7.713	21.131	0.65	15.14	C	
	ATOM	4124	OD1B	AASP	B	51	-6.122	-9.033	20.747	0.35	14.80	O	
	ATOM	4125	OD1A	AASP	B	51	-5.098	-7.505	20.497	0.65	16.90	O	
	ATOM	4126	OD2B	AASP	B	51	-6.018	-6.909	20.468	0.35	15.44	O	
5	ATOM	4127	OD2A	AASP	B	51	-7.200	-7.900	20.492	0.65	16.43	O	
	ATOM	4128	C	ASP	B	51	-8.601	-7.577	22.843	1.00	11.68	C	
	ATOM	4129	O	ASP	B	51	-8.809	-8.770	23.089	1.00	12.14	O	
	ATOM	4130	N	THR	B	52	-9.484	-6.811	22.224	1.00	12.82	N	
	ATOM	4132	CA	THR	B	52	-10.821	-7.311	21.944	1.00	14.29	C	
10	ATOM	4134	CB	THR	B	52	-11.794	-6.158	21.621	1.00	15.31	C	
	ATOM	4136	OG1	THR	B	52	-11.342	-5.436	20.473	1.00	17.85	O	
	ATOM	4138	CG2	THR	B	52	-11.813	-5.133	22.748	1.00	15.84	C	
	ATOM	4142	C	THR	B	52	-10.849	-8.374	20.842	1.00	15.07	C	
	ATOM	4143	O	THR	B	52	-11.736	-9.221	20.836	1.00	16.91	O	
15	ATOM	4144	N	SER	B	53	-9.900	-8.338	19.911	1.00	15.21	N	
	ATOM	4146	CA	SER	B	53	-9.869	-9.326	18.824	1.00	15.87	C	
	ATOM	4148	CB	B	SER	B	53	-8.908	-8.886	17.708	0.35	16.21	C
	ATOM	4149	CB	A	SER	B	53	-8.859	-8.903	17.756	0.65	16.72	C
	ATOM	4154	OG	B	SER	B	53	-7.569	-8.772	18.157	0.35	17.00	O
20	ATOM	4155	OG	A	SER	B	53	-8.752	-9.892	16.748	0.65	18.99	O
	ATOM	4158	C	SER	B	53	-9.530	-10.736	19.309	1.00	15.03	C	
	ATOM	4159	O	SER	B	53	-10.178	-11.722	18.919	1.00	14.93	O	
	ATOM	4160	N	SER	B	54	-8.511	-10.836	20.153	1.00	14.11	N	
	ATOM	4162	CA	SER	B	54	-8.082	-12.117	20.691	1.00	13.76	C	
25	ATOM	4164	CB	SER	B	54	-6.585	-12.082	20.984	1.00	14.63	C	
	ATOM	4167	OG	SER	B	54	-6.302	-11.212	22.069	1.00	15.48	O	
	ATOM	4169	C	SER	B	54	-8.830	-12.497	21.955	1.00	12.67	C	
	ATOM	4170	O	SER	B	54	-8.716	-13.624	22.416	1.00	13.34	O	
	ATOM	4171	N	GLY	B	55	-9.564	-11.539	22.518	1.00	12.60	N	
30	ATOM	4173	CA	GLY	B	55	-10.337	-11.766	23.724	1.00	12.43	C	
	ATOM	4176	C	GLY	B	55	-9.474	-11.987	24.936	1.00	11.80	C	
	ATOM	4177	O	GLY	B	55	-9.834	-12.737	25.833	1.00	12.09	O	
	ATOM	4178	N	SER	B	56	-8.333	-11.313	24.993	1.00	12.30	N	
	ATOM	4180	CA	SER	B	56	-7.404	-11.563	26.071	1.00	12.22	C	
35	ATOM	4182	CB	SER	B	56	-6.277	-12.470	25.600	1.00	13.33	C	
	ATOM	4185	OG	SER	B	56	-5.511	-11.840	24.607	1.00	17.47	O	
	ATOM	4187	C	SER	B	56	-6.813	-10.288	26.619	1.00	10.81	C	
	ATOM	4188	O	SER	B	56	-6.567	-9.310	25.907	1.00	10.50	O	
	ATOM	4189	N	PHE	B	57	-6.573	-10.325	27.916	1.00	9.99	N	
40	ATOM	4191	CA	PHE	B	57	-5.790	-9.301	28.562	1.00	9.43	C	
	ATOM	4193	CB	PHE	B	57	-5.887	-9.455	30.080	1.00	10.07	C	
	ATOM	4196	CG	PHE	B	57	-7.232	-9.069	30.620	1.00	10.41	C	
	ATOM	4197	CD1	PHE	B	57	-7.527	-7.744	30.869	1.00	10.08	C	
	ATOM	4199	CE1	PHE	B	57	-8.774	-7.363	31.333	1.00	11.19	C	
45	ATOM	4201	CZ	PHE	B	57	-9.751	-8.313	31.532	1.00	12.88	C	
	ATOM	4203	CE2	PHE	B	57	-9.476	-9.645	31.264	1.00	13.00	C	
	ATOM	4205	CD2	PHE	B	57	-8.230	-10.020	30.810	1.00	12.20	C	
	ATOM	4207	C	PHE	B	57	-4.347	-9.410	28.102	1.00	9.19	C	
	ATOM	4208	O	PHE	B	57	-3.877	-10.475	27.678	1.00	10.24	O	
50	ATOM	4209	N	ALA	B	58	-3.643	-8.288	28.189	1.00	9.20	N	
	ATOM	4211	CA	ALA	B	58	-2.202	-8.292	28.075	1.00	9.09	C	
	ATOM	4213	CB	ALA	B	58	-1.664	-6.887	28.322	1.00	9.63	C	
	ATOM	4217	C	ALA	B	58	-1.601	-9.247	29.090	1.00	9.25	C	
	ATOM	4218	O	ALA	B	58	-2.213	-9.573	30.105	1.00	9.38	O	
55	ATOM	4219	N	GLY	B	59	-0.371	-9.666	28.838	1.00	9.59	N	
	ATOM	4221	CA	GLY	B	59	0.444	-10.276	29.857	1.00	9.95	C	
	ATOM	4224	C	GLY	B	59	0.793	-9.242	30.908	1.00	9.76	C	
	ATOM	4225	O	GLY	B	59	0.308	-8.099	30.891	1.00	10.29	O	
	ATOM	4226	N	THR	B	60	1.637	-9.646	31.834	1.00	10.02	N	
60	ATOM	4228	CA	THR	B	60	2.060	-8.759	32.898	1.00	10.25	C	
	ATOM	4230	CB	THR	B	60	3.107	-9.463	33.740	1.00	11.48	C	
	ATOM	4232	OG1	THR	B	60	2.519	-10.662	34.262	1.00	13.35	O	
	ATOM	4234	CG2	THR	B	60	3.526	-8.622	34.941	1.00	12.09	C	
	ATOM	4238	C	THR	B	60	2.629	-7.471	32.338	1.00	9.81	C	

	ATOM	4239	O	THR	B	60	3.465	-7.498	31.441	1.00	10.64	O
	ATOM	4240	N	ALA	B	61	2.176	-6.351	32.884	1.00	9.32	N
	ATOM	4242	CA	ALA	B	61	2.677	-5.044	32.503	1.00	9.32	C
5	ATOM	4244	CB	ALA	B	61	1.568	-3.981	32.587	1.00	9.62	C
	ATOM	4248	C	ALA	B	61	3.837	-4.632	33.385	1.00	8.92	C
	ATOM	4249	O	ALA	B	61	3.876	-4.954	34.567	1.00	10.09	O
	ATOM	4250	N	THR	B	62	4.756	-3.882	32.793	1.00	9.06	N
	ATOM	4252	CA	THR	B	62	5.844	-3.224	33.497	1.00	9.56	C
	ATOM	4254	CB	THR	B	62	7.159	-3.456	32.762	1.00	10.57	C
10	ATOM	4256	OG1	THR	B	62	7.423	-4.870	32.721	1.00	11.83	O
	ATOM	4258	CG2	THR	B	62	8.326	-2.808	33.497	1.00	12.14	C
	ATOM	4262	C	THR	B	62	5.495	-1.745	33.556	1.00	8.59	C
	ATOM	4263	O	THR	B	62	5.334	-1.089	32.521	1.00	9.17	O
	ATOM	4264	N	VAL	B	63	5.359	-1.225	34.771	1.00	8.26	N
15	ATOM	4266	CA	VAL	B	63	4.826	0.118	35.013	1.00	8.04	C
	ATOM	4268	CB	VAL	B	63	3.546	0.039	35.861	1.00	8.65	C
	ATOM	4270	CG1	VAL	B	63	3.023	1.431	36.176	1.00	9.71	C
	ATOM	4274	CG2	VAL	B	63	2.478	-0.794	35.150	1.00	9.51	C
	ATOM	4278	C	VAL	B	63	5.891	0.959	35.693	1.00	7.95	C
20	ATOM	4279	O	VAL	B	63	6.369	0.597	36.771	1.00	8.82	O
	ATOM	4280	N	SER	B	64	6.254	2.083	35.085	1.00	7.68	N
	ATOM	4282	CA	SER	B	64	7.393	2.863	35.515	1.00	8.03	C
	ATOM	4284	CB	SER	B	64	8.499	2.805	34.462	1.00	8.70	C
	ATOM	4287	OG	SER	B	64	8.898	1.469	34.228	1.00	9.66	O
25	ATOM	4289	C	SER	B	64	6.965	4.306	35.757	1.00	7.95	C
	ATOM	4290	O	SER	B	64	6.893	5.116	34.823	1.00	7.83	O
	ATOM	4291	N	PRO	B	65	6.648	4.658	37.004	1.00	8.11	N
	ATOM	4292	CA	PRO	B	65	6.226	6.028	37.301	1.00	8.10	C
	ATOM	4294	CB	PRO	B	65	5.859	5.970	38.795	1.00	8.49	C
30	ATOM	4297	CG	PRO	B	65	5.584	4.520	39.054	1.00	8.49	C
	ATOM	4300	CD	PRO	B	65	6.600	3.807	38.204	1.00	8.68	C
	ATOM	4303	C	PRO	B	65	7.344	7.027	37.057	1.00	8.00	C
	ATOM	4304	O	PRO	B	65	8.483	6.807	37.481	1.00	8.46	O
	ATOM	4305	N	GLY	B	66	7.038	8.127	36.383	1.00	7.75	N
35	ATOM	4307	CA	GLY	B	66	8.034	9.166	36.186	1.00	8.40	C
	ATOM	4310	C	GLY	B	66	9.266	8.699	35.428	1.00	8.24	C
	ATOM	4311	O	GLY	B	66	10.346	9.265	35.586	1.00	8.86	O
	ATOM	4312	N	ARG	B	67	9.123	7.685	34.585	1.00	8.08	N
	ATOM	4314	CA	ARG	B	67	10.223	7.252	33.745	1.00	8.11	C
40	ATOM	4316	CB	ARG	B	67	9.753	6.160	32.802	1.00	8.27	C
	ATOM	4319	CG	ARG	B	67	10.864	5.568	31.971	1.00	8.88	C
	ATOM	4322	CD	ARG	B	67	10.435	4.444	31.086	1.00	8.89	C
	ATOM	4325	NE	ARG	B	67	11.498	4.135	30.142	1.00	9.16	N
	ATOM	4327	CZ	ARG	B	67	11.404	3.282	29.149	1.00	10.30	C
45	ATOM	4328	NH1	ARG	B	67	12.410	3.169	28.296	1.00	11.25	N
	ATOM	4331	NH2	ARG	B	67	10.320	2.541	29.004	1.00	12.36	N
	ATOM	4334	C	ARG	B	67	10.750	8.429	32.946	1.00	8.11	C
	ATOM	4335	O	ARG	B	67	9.983	9.254	32.462	1.00	8.22	O
	ATOM	4336	N	ASN	B	68	12.070	8.472	32.783	1.00	8.17	N
50	ATOM	4338	CA	ASN	B	68	12.720	9.484	31.970	1.00	8.77	C
	ATOM	4340	CB	ASN	B	68	13.312	10.573	32.848	1.00	9.40	C
	ATOM	4343	CG	ASN	B	68	13.931	11.660	32.023	1.00	10.39	C
	ATOM	4344	OD1	ASN	B	68	13.349	12.050	31.010	1.00	11.79	O
	ATOM	4345	ND2	ASN	B	68	15.136	12.110	32.385	1.00	12.51	N
55	ATOM	4348	C	ASN	B	68	13.812	8.863	31.104	1.00	9.03	C
	ATOM	4349	O	ASN	B	68	14.994	8.879	31.455	1.00	9.74	O
	ATOM	4350	N	GLY	B	69	13.405	8.293	29.977	1.00	9.60	N
	ATOM	4352	CA	GLY	B	69	14.329	7.682	29.037	1.00	9.82	C
	ATOM	4355	C	GLY	B	69	14.763	6.335	29.549	1.00	9.55	C
60	ATOM	4356	O	GLY	B	69	13.946	5.419	29.628	1.00	10.48	O
	ATOM	4357	N	THR	B	70	16.040	6.194	29.885	1.00	9.50	N
	ATOM	4359	CA	THR	B	70	16.516	4.977	30.529	1.00	10.01	C
	ATOM	4361	CB	THR	B	70	17.775	4.427	29.839	1.00	10.64	C
	ATOM	4363	OG1	THR	B	70	18.745	5.471	29.679	1.00	11.68	O

5	ATOM	4365	CG2	THR	B	70	17.437	3.934	28.436	1.00	11.69	C
	ATOM	4369	C	THR	B	70	16.747	5.185	32.024	1.00	10.48	C
	ATOM	4370	O	THR	B	70	17.362	4.357	32.689	1.00	11.63	O
	ATOM	4371	N	SER	B	71	16.214	6.274	32.558	1.00	10.58	N
	ATOM	4373	CA	SER	B	71	16.175	6.510	33.992	1.00	10.32	C
	ATOM	4375	CB	SER	B	71	16.437	7.969	34.309	1.00	10.45	C
	ATOM	4378	OG	SER	B	71	17.669	8.393	33.780	1.00	11.02	O
	ATOM	4380	C	SER	B	71	14.821	6.139	34.562	1.00	9.95	C
10	ATOM	4381	O	SER	B	71	13.775	6.496	34.006	1.00	10.07	O
	ATOM	4382	N	TYR	B	72	14.853	5.470	35.710	1.00	9.77	N
	ATOM	4384	CA	TYR	B	72	13.665	4.953	36.370	1.00	9.83	C
	ATOM	4386	CB	TYR	B	72	13.637	3.420	36.298	1.00	10.11	C
15	ATOM	4389	CG	TYR	B	72	13.491	2.884	34.890	1.00	10.38	C
	ATOM	4390	CD1	TYR	B	72	12.261	2.467	34.422	1.00	10.73	C
	ATOM	4392	CE1	TYR	B	72	12.112	1.963	33.142	1.00	11.24	C
	ATOM	4394	CZ	TYR	B	72	13.200	1.895	32.301	1.00	11.23	C
	ATOM	4395	OH	TYR	B	72	13.014	1.381	31.041	1.00	12.73	O
	ATOM	4397	CE2	TYR	B	72	14.442	2.316	32.741	1.00	11.74	C
	ATOM	4399	CD2	TYR	B	72	14.581	2.804	34.018	1.00	11.15	C
	ATOM	4401	C	TYR	B	72	13.739	5.443	37.815	1.00	10.00	C
20	ATOM	4402	O	TYR	B	72	14.125	4.683	38.712	1.00	10.50	O
	ATOM	4403	N	PRO	B	73	13.426	6.715	38.070	1.00	10.19	N
	ATOM	4404	CA	PRO	B	73	13.605	7.254	39.425	1.00	10.57	C
	ATOM	4406	CB	PRO	B	73	13.195	8.719	39.285	1.00	10.64	C
	ATOM	4409	CG	PRO	B	73	12.351	8.766	38.059	1.00	10.69	C
	ATOM	4412	CD	PRO	B	73	12.927	7.742	37.134	1.00	10.07	C
	ATOM	4415	C	PRO	B	73	12.778	6.561	40.497	1.00	10.59	C
	ATOM	4416	O	PRO	B	73	13.139	6.627	41.664	1.00	12.17	O
30	ATOM	4417	N	TYR	B	74	11.692	5.916	40.097	1.00	10.13	N
	ATOM	4419	CA	TYR	B	74	10.834	5.165	41.004	1.00	10.86	C
	ATOM	4421	CB	TYR	B	74	9.425	5.767	41.038	1.00	10.82	C
	ATOM	4424	CG	TYR	B	74	9.500	7.222	41.399	1.00	10.36	C
	ATOM	4425	CD1	TYR	B	74	9.391	8.194	40.416	1.00	10.82	C
	ATOM	4427	CE1	TYR	B	74	9.519	9.518	40.701	1.00	11.59	C
	ATOM	4429	CZ	TYR	B	74	9.748	9.915	41.996	1.00	11.91	C
	ATOM	4430	OH	TYR	B	74	9.863	11.261	42.253	1.00	14.01	O
40	ATOM	4432	CE2	TYR	B	74	9.864	8.972	43.005	1.00	12.35	C
	ATOM	4434	CD2	TYR	B	74	9.752	7.632	42.700	1.00	11.52	C
	ATOM	4436	C	TYR	B	74	10.788	3.696	40.635	1.00	11.39	C
	ATOM	4437	O	TYR	B	74	9.849	2.993	41.013	1.00	12.84	O
	ATOM	4438	N	GLY	B	75	11.820	3.222	39.939	1.00	10.85	N
	ATOM	4440	CA	GLY	B	75	11.872	1.851	39.479	1.00	10.79	C
	ATOM	4443	C	GLY	B	75	10.764	1.505	38.505	1.00	10.56	C
	ATOM	4444	O	GLY	B	75	10.129	2.370	37.891	1.00	10.85	O
45	ATOM	4445	N	SER	B	76	10.563	0.202	38.377	1.00	10.90	N
	ATOM	4447	CA	SER	B	76	9.489	-0.367	37.607	1.00	11.52	C
	ATOM	4449	CB	BSER	B	76	10.053	-1.085	36.386	0.35	11.19	C
	ATOM	4450	CB	ASER	B	76	9.998	-0.975	36.309	0.65	13.32	C
	ATOM	4455	OG	BSER	B	76	10.704	-0.188	35.508	0.35	7.99	O
	ATOM	4456	OG	ASER	B	76	10.880	-2.042	36.529	0.65	17.36	O
	ATOM	4459	C	SER	B	76	8.802	-1.393	38.474	1.00	11.22	C
	ATOM	4460	O	SER	B	76	9.444	-2.102	39.264	1.00	12.58	O
55	ATOM	4461	N	VAL	B	77	7.489	-1.472	38.325	1.00	10.56	N
	ATOM	4463	CA	VAL	B	77	6.668	-2.352	39.116	1.00	10.65	C
	ATOM	4465	CB	BVAL	B	77	5.793	-1.531	40.080	0.35	10.56	C
	ATOM	4466	CB	AVAL	B	77	5.843	-1.555	40.151	0.65	11.99	C
	ATOM	4469	CG1BVAL	B	77	4.837	-2.397	40.810	0.35	8.39	C	
	ATOM	4470	CG1AVAL	B	77	6.704	-0.441	40.775	0.65	12.64	C	
	ATOM	4477	CG2BVAL	B	77	6.661	-0.843	41.119	0.35	11.33	C	
	ATOM	4478	CG2AVAL	B	77	4.627	-0.943	39.578	0.65	12.49	C	
60	ATOM	4485	C	VAL	B	77	5.801	-3.183	38.174	1.00	9.91	C
	ATOM	4486	O	VAL	B	77	5.303	-2.699	37.163	1.00	12.13	O
	ATOM	4487	N	LYS	B	78	5.596	-4.440	38.500	1.00	11.93	N
	ATOM	4489	CA	LYS	B	78	4.790	-5.315	37.664	1.00	12.70	C

	ATOM	4491	CB	LYS	B	78	5.236	-6.767	37.809	1.00	13.36	C
	ATOM	4494	CG	LYS	B	78	6.666	-7.034	37.399	1.00	15.87	C
	ATOM	4497	CD	LYS	B	78	6.906	-6.736	35.938	1.00	17.52	C
5	ATOM	4500	CE	LYS	B	78	8.294	-7.176	35.540	1.00	19.94	C
	ATOM	4503	NZ	LYS	B	78	8.671	-6.739	34.188	1.00	22.53	N
	ATOM	4507	C	LYS	B	78	3.338	-5.215	38.065	1.00	12.78	C
	ATOM	4508	O	LYS	B	78	3.035	-5.045	39.243	1.00	14.43	O
	ATOM	4509	N	SER	B	79	2.436	-5.360	37.098	1.00	12.03	N
10	ATOM	4511	CA	SER	B	79	1.017	-5.509	37.378	1.00	11.74	C
	ATOM	4513	CB	SER	B	79	0.196	-5.437	36.090	1.00	11.51	C
	ATOM	4516	OG	SER	B	79	0.508	-6.477	35.178	1.00	10.77	O
	ATOM	4518	C	SER	B	79	0.722	-6.833	38.044	1.00	11.16	C
	ATOM	4519	O	SER	B	79	1.441	-7.826	37.856	1.00	12.07	O
15	ATOM	4520	N	THR	B	80	-0.360	-6.849	38.804	1.00	10.75	N
	ATOM	4522	CA	THR	B	80	-0.923	-8.093	39.298	1.00	11.21	C
	ATOM	4524	CB	THR	B	80	-1.164	-8.015	40.807	1.00	11.49	C
	ATOM	4526	OG1	THR	B	80	-1.989	-6.887	41.124	1.00	12.77	O
	ATOM	4528	CG2	THR	B	80	0.154	-7.823	41.547	1.00	11.88	C
20	ATOM	4532	C	THR	B	80	-2.196	-8.485	38.578	1.00	11.33	C
	ATOM	4533	O	THR	B	80	-2.490	-9.682	38.478	1.00	12.80	O
	ATOM	4534	N	ARG	B	81	-2.959	-7.489	38.114	1.00	11.02	N
	ATOM	4536	CA	ARG	B	81	-4.210	-7.752	37.427	1.00	10.96	C
	ATOM	4538	CB	ARG	B	81	-5.240	-8.338	38.374	1.00	11.73	C
25	ATOM	4541	CG	ARG	B	81	-5.626	-7.375	39.459	1.00	11.26	C
	ATOM	4544	CD	ARG	B	81	-6.558	-7.993	40.419	1.00	13.29	C
	ATOM	4547	NE	ARG	B	81	-6.874	-7.102	41.525	1.00	14.74	N
	ATOM	4549	CZ	ARG	B	81	-7.891	-7.291	42.357	1.00	13.37	C
	ATOM	4550	NH1	ARG	B	81	-8.139	-6.424	43.336	1.00	11.04	N
	ATOM	4553	NH2	ARG	B	81	-8.704	-8.320	42.185	1.00	16.83	N
30	ATOM	4556	C	ARG	B	81	-4.748	-6.458	36.824	1.00	9.86	C
	ATOM	4557	O	ARG	B	81	-4.234	-5.348	37.074	1.00	10.32	O
	ATOM	4558	N	TYR	B	82	-5.781	-6.619	36.013	1.00	9.05	N
	ATOM	4560	CA	TYR	B	82	-6.392	-5.564	35.243	1.00	8.45	C
35	ATOM	4562	CB	TYR	B	82	-6.236	-5.882	33.761	1.00	8.46	C
	ATOM	4565	CG	TYR	B	82	-4.815	-5.913	33.273	1.00	8.62	C
	ATOM	4566	CD1	TYR	B	82	-4.012	-4.791	33.367	1.00	9.06	C
	ATOM	4568	CE1	TYR	B	82	-2.711	-4.804	32.888	1.00	9.18	C
	ATOM	4570	CZ	TYR	B	82	-2.202	-5.950	32.310	1.00	8.79	C
40	ATOM	4571	OH	TYR	B	82	-0.907	-5.894	31.850	1.00	9.78	O
	ATOM	4573	CE2	TYR	B	82	-2.990	-7.081	32.209	1.00	9.01	C
	ATOM	4575	CD2	TYR	B	82	-4.284	-7.053	32.688	1.00	9.11	C
	ATOM	4577	C	TYR	B	82	-7.886	-5.476	35.560	1.00	8.75	C
	ATOM	4578	O	TYR	B	82	-8.513	-6.470	35.949	1.00	9.58	O
45	ATOM	4579	N	PHE	B	83	-8.447	-4.290	35.362	1.00	8.61	N
	ATOM	4581	CA	PHE	B	83	-9.874	-4.032	35.444	1.00	8.66	C
	ATOM	4583	CB	PHE	B	83	-10.228	-3.092	36.585	1.00	9.00	C
	ATOM	4586	CG	PHE	B	83	-9.748	-3.516	37.936	1.00	9.24	C
	ATOM	4587	CD1	PHE	B	83	-8.475	-3.177	38.366	1.00	10.26	C
	ATOM	4589	CE1	PHE	B	83	-8.059	-3.502	39.639	1.00	11.44	C
50	ATOM	4591	CZ	PHE	B	83	-8.911	-4.173	40.501	1.00	12.62	C
	ATOM	4593	CE2	PHE	B	83	-10.177	-4.495	40.104	1.00	12.07	C
	ATOM	4595	CD2	PHE	B	83	-10.604	-4.166	38.823	1.00	10.68	C
	ATOM	4597	C	PHE	B	83	-10.298	-3.339	34.160	1.00	8.76	C
	ATOM	4598	O	PHE	B	83	-9.630	-2.409	33.699	1.00	8.65	O
55	ATOM	4599	N	ILE	B	84	-11.421	-3.768	33.598	1.00	8.84	N
	ATOM	4601	CA	ILE	B	84	-12.048	-3.068	32.478	1.00	9.12	C
	ATOM	4603	CB	ILE	B	84	-11.734	-3.740	31.118	1.00	9.53	C
	ATOM	4605	CG1	ILE	B	84	-12.103	-5.225	31.124	1.00	10.28	C
	ATOM	4608	CD1	ILE	B	84	-11.973	-5.909	29.791	1.00	12.10	C
60	ATOM	4612	CG2	ILE	B	84	-10.281	-3.522	30.746	1.00	10.12	C
	ATOM	4616	C	ILE	B	84	-13.552	-3.018	32.691	1.00	8.87	C
	ATOM	4617	O	ILE	B	84	-14.134	-3.904	33.327	1.00	9.55	O
	ATOM	4618	N	PRO	B	85	-14.198	-2.004	32.131	1.00	9.08	N
	ATOM	4619	CA	PRO	B	85	-15.660	-1.982	32.154	1.00	9.52	C

	ATOM	4621	CB	PRO	B	85	-15.984	-0.561	31.686	1.00	10.01	C
	ATOM	4624	CG	PRO	B	85	-14.849	-0.235	30.745	1.00	9.80	C
	ATOM	4627	CD	PRO	B	85	-13.642	-0.866	31.371	1.00	9.00	C
5	ATOM	4630	C	PRO	B	85	-16.212	-3.010	31.176	1.00	10.10	C
	ATOM	4631	O	PRO	B	85	-15.561	-3.364	30.210	1.00	10.32	O
	ATOM	4632	N	SER	B	86	-17.437	-3.459	31.407	1.00	11.25	N
	ATOM	4634	CA	SER	B	86	-18.073	-4.415	30.502	1.00	12.53	C
	ATOM	4636	CB	BSER	B	86	-19.506	-4.715	30.963	0.35	13.18	C
	ATOM	4637	CB	ASER	B	86	-19.476	-4.789	30.986	0.65	13.91	C
10	ATOM	4642	OG	BSER	B	86	-19.544	-5.098	32.327	0.35	14.94	O
	ATOM	4643	OG	ASER	B	86	-20.279	-3.644	31.135	0.65	17.06	O
	ATOM	4646	C	SER	B	86	-18.116	-3.886	29.071	1.00	12.33	C
	ATOM	4647	O	SER	B	86	-17.957	-4.654	28.127	1.00	13.62	O
	ATOM	4648	N	GLY	B	87	-18.305	-2.578	28.911	1.00	12.00	N
15	ATOM	4650	CA	GLY	B	87	-18.365	-1.984	27.589	1.00	12.48	C
	ATOM	4653	C	GLY	B	87	-17.076	-2.129	26.808	1.00	12.31	C
	ATOM	4654	O	GLY	B	87	-17.114	-2.175	25.583	1.00	14.16	O
	ATOM	4655	N	TRP	B	88	-15.931	-2.192	27.495	1.00	11.78	N
	ATOM	4657	CA	TRP	B	88	-14.658	-2.408	26.804	1.00	11.93	C
20	ATOM	4659	CB	TRP	B	88	-13.432	-1.778	27.501	1.00	11.63	C
	ATOM	4662	CG	TRP	B	88	-12.253	-1.984	26.598	1.00	10.61	C
	ATOM	4663	CD1	TRP	B	88	-11.202	-2.843	26.769	1.00	10.04	C
	ATOM	4665	NE1	TRP	B	88	-10.404	-2.843	25.652	1.00	10.00	N
	ATOM	4667	CE2	TRP	B	88	-10.932	-1.976	24.733	1.00	9.81	C
25	ATOM	4668	CD2	TRP	B	88	-12.106	-1.434	25.292	1.00	10.13	C
	ATOM	4669	CE3	TRP	B	88	-12.838	-0.519	24.539	1.00	11.31	C
	ATOM	4671	CZ3	TRP	B	88	-12.403	-0.194	23.276	1.00	12.74	C
	ATOM	4673	CH2	TRP	B	88	-11.247	-0.752	22.749	1.00	12.39	C
	ATOM	4675	CZ2	TRP	B	88	-10.504	-1.659	23.451	1.00	11.10	C
30	ATOM	4677	C	TRP	B	88	-14.384	-3.874	26.554	1.00	13.20	C
	ATOM	4678	O	TRP	B	88	-13.795	-4.228	25.544	1.00	14.61	O
	ATOM	4679	N	ARG	B	89	-14.818	-4.742	27.456	1.00	14.97	N
	ATOM	4681	CA	ARG	B	89	-14.786	-6.157	27.135	1.00	17.45	C
	ATOM	4683	CB	ARG	B	89	-15.489	-6.978	28.216	1.00	18.59	C
35	ATOM	4686	CG	ARG	B	89	-14.972	-8.407	28.352	1.00	20.17	C
	ATOM	4689	CD	ARG	B	89	-15.609	-9.163	29.496	1.00	22.60	C
	ATOM	4692	NE	ARG	B	89	-15.033	-8.796	30.790	1.00	24.39	N
	ATOM	4694	CZ	ARG	B	89	-13.948	-9.349	31.330	1.00	25.64	C
	ATOM	4695	NH1	ARG	B	89	-13.279	-10.314	30.701	1.00	26.15	N
40	ATOM	4698	NH2	ARG	B	89	-13.524	-8.931	32.516	1.00	26.48	N
	ATOM	4701	C	ARG	B	89	-15.423	-6.339	25.731	1.00	19.17	C
	ATOM	4702	O	ARG	B	89	-15.043	-7.254	24.999	1.00	20.41	O
	ATOM	4703	N	SER	B	90	-16.345	-5.436	25.357	1.00	20.72	N
	ATOM	4705	CA	SER	B	90	-16.995	-5.405	24.034	1.00	21.67	C
45	ATOM	4707	CB	SER	B	90	-18.412	-4.837	24.189	1.00	22.17	C
	ATOM	4710	OG	SER	B	90	-19.158	-5.584	25.125	1.00	23.91	O
	ATOM	4712	C	SER	B	90	-16.267	-4.630	22.917	1.00	21.74	C
	ATOM	4713	O	SER	B	90	-16.614	-4.789	21.746	1.00	23.22	O
	ATOM	4714	N	GLY	B	91	-15.307	-3.771	23.253	1.00	21.01	N
50	ATOM	4716	CA	GLY	B	91	-14.547	-3.027	22.258	1.00	20.45	C
	ATOM	4719	C	GLY	B	91	-15.224	-1.724	21.881	1.00	20.20	C
	ATOM	4720	O	GLY	B	91	-14.868	-1.062	20.893	1.00	20.84	O
	ATOM	4721	N	ASN	B	92	-16.222	-1.355	22.669	1.00	19.28	N
	ATOM	4723	CA	ASN	B	92	-16.957	-0.132	22.417	1.00	18.38	C
55	ATOM	4725	CB	ASN	B	92	-18.294	-0.171	23.169	1.00	17.92	C
	ATOM	4728	CG	ASN	B	92	-19.210	0.960	22.777	1.00	17.67	C
	ATOM	4729	OD1	ASN	B	92	-18.749	2.066	22.576	1.00	15.73	O
	ATOM	4730	ND2	ASN	B	92	-20.510	0.686	22.670	1.00	20.42	N
	ATOM	4733	C	ASN	B	92	-16.081	1.052	22.845	1.00	17.91	C
60	ATOM	4734	O	ASN	B	92	-15.757	1.155	24.021	1.00	17.12	O
	ATOM	4735	N	THR	B	93	-15.667	1.909	21.902	1.00	17.67	N
	ATOM	4737	CA	THR	B	93	-14.789	3.068	22.178	1.00	17.77	C
	ATOM	4739	CB	THR	B	93	-14.457	3.902	20.885	1.00	18.92	C
	ATOM	4741	OG1	THR	B	93	-13.708	5.097	21.206	1.00	21.52	O

	ATOM	4743	CG2	THR	B	93	-15.718	4.434	20.237	1.00	18.95	C
	ATOM	4747	C	THR	B	93	-15.348	4.006	23.212	1.00	15.15	C
	ATOM	4748	O	THR	B	93	-14.591	4.719	23.860	1.00	14.57	O
5	ATOM	4749	N	ASN	B	94	-16.676	4.042	23.349	1.00	13.37	N
	ATOM	4751	CA	ASN	B	94	-17.263	4.874	24.369	1.00	11.96	C
	ATOM	4753	CB	ASN	B	94	-18.767	5.001	24.180	1.00	12.29	C
	ATOM	4756	CG	ASN	B	94	-19.122	5.919	23.041	1.00	14.50	C
	ATOM	4757	OD1	ASN	B	94	-18.348	6.785	22.653	1.00	16.90	O
	ATOM	4758	ND2	ASN	B	94	-20.312	5.739	22.508	1.00	17.07	N
10	ATOM	4761	C	ASN	B	94	-16.951	4.400	25.772	1.00	10.32	C
	ATOM	4762	O	ASN	B	94	-17.229	5.130	26.707	1.00	10.75	O
	ATOM	4763	N	TYR	B	95	-16.361	3.207	25.915	1.00	9.75	N
	ATOM	4765	CA	TYR	B	95	-15.992	2.654	27.219	1.00	9.39	C
	ATOM	4767	CB	TYR	B	95	-16.876	1.444	27.541	1.00	10.00	C
15	ATOM	4770	CG	TYR	B	95	-18.334	1.826	27.578	1.00	10.15	C
	ATOM	4771	CD1	TYR	B	95	-19.127	1.734	26.446	1.00	11.72	C
	ATOM	4773	CE1	TYR	B	95	-20.466	2.105	26.467	1.00	12.50	C
	ATOM	4775	CZ	TYR	B	95	-21.008	2.602	27.625	1.00	13.24	C
	ATOM	4776	OH	TYR	B	95	-22.332	2.984	27.661	1.00	14.96	O
20	ATOM	4778	CE2	TYR	B	95	-20.243	2.720	28.762	1.00	12.98	C
	ATOM	4780	CD2	TYR	B	95	-18.911	2.333	28.733	1.00	11.90	C
	ATOM	4782	C	TYR	B	95	-14.512	2.285	27.263	1.00	8.95	C
	ATOM	4783	O	TYR	B	95	-14.114	1.400	28.010	1.00	8.99	O
	ATOM	4784	N	ASP	B	96	-13.695	2.996	26.485	1.00	8.73	N
25	ATOM	4786	CA	ASP	B	96	-12.272	2.693	26.401	1.00	8.57	C
	ATOM	4788	CB	ASP	B	96	-11.716	3.169	25.067	1.00	8.34	C
	ATOM	4791	CG	ASP	B	96	-10.298	2.720	24.829	1.00	8.28	C
	ATOM	4792	OD1	ASP	B	96	-9.773	3.069	23.732	1.00	8.57	O
	ATOM	4793	OD2	ASP	B	96	-9.674	2.040	25.677	1.00	8.50	O
30	ATOM	4794	C	ASP	B	96	-11.510	3.314	27.580	1.00	7.91	C
	ATOM	4795	O	ASP	B	96	-11.002	4.442	27.510	1.00	8.32	O
	ATOM	4796	N	TYR	B	97	-11.479	2.567	28.671	1.00	8.03	N
	ATOM	4798	CA	TYR	B	97	-10.719	2.910	29.860	1.00	7.75	C
	ATOM	4800	CB	TYR	B	97	-11.386	3.992	30.707	1.00	7.83	C
35	ATOM	4803	CG	TYR	B	97	-12.688	3.607	31.371	1.00	8.16	C
	ATOM	4804	CD1	TYR	B	97	-13.893	3.674	30.681	1.00	8.43	C
	ATOM	4806	CE1	TYR	B	97	-15.092	3.350	31.297	1.00	8.51	C
	ATOM	4808	CZ	TYR	B	97	-15.094	2.959	32.628	1.00	8.68	C
	ATOM	4809	OH	TYR	B	97	-16.265	2.673	33.298	1.00	9.75	O
40	ATOM	4811	CE2	TYR	B	97	-13.906	2.878	33.321	1.00	8.95	C
	ATOM	4813	CD2	TYR	B	97	-12.719	3.205	32.697	1.00	8.55	C
	ATOM	4815	C	TYR	B	97	-10.531	1.625	30.653	1.00	7.47	C
	ATOM	4816	O	TYR	B	97	-11.168	0.607	30.383	1.00	7.98	O
	ATOM	4817	N	GLY	B	98	-9.659	1.684	31.647	1.00	7.59	N
45	ATOM	4819	CA	GLY	B	98	-9.409	0.542	32.502	1.00	7.74	C
	ATOM	4822	C	GLY	B	98	-8.451	0.917	33.603	1.00	7.36	C
	ATOM	4823	O	GLY	B	98	-8.061	2.082	33.748	1.00	7.93	O
	ATOM	4824	N	ALA	B	99	-8.079	-0.080	34.390	1.00	7.67	N
	ATOM	4826	CA	ALA	B	99	-7.139	0.136	35.465	1.00	7.61	C
50	ATOM	4828	CB	ALA	B	99	-7.846	0.428	36.770	1.00	8.42	C
	ATOM	4832	C	ALA	B	99	-6.207	-1.042	35.626	1.00	8.10	C
	ATOM	4833	O	ALA	B	99	-6.523	-2.172	35.222	1.00	8.34	O
	ATOM	4834	N	ILE	B	100	-5.045	-0.762	36.211	1.00	8.17	N
	ATOM	4836	CA	ILE	B	100	-4.042	-1.770	36.490	1.00	8.36	C
55	ATOM	4838	CB	ILE	B	100	-2.709	-1.485	35.749	1.00	8.61	C
	ATOM	4840	CG1	ILE	B	100	-2.941	-1.193	34.265	1.00	8.93	C
	ATOM	4843	CD1	ILE	B	100	-1.682	-0.873	33.485	1.00	10.13	C
	ATOM	4847	CG2	ILE	B	100	-1.738	-2.640	35.958	1.00	9.23	C
	ATOM	4851	C	ILE	B	100	-3.764	-1.741	37.982	1.00	8.16	C
60	ATOM	4852	O	ILE	B	100	-3.527	-0.682	38.549	1.00	8.74	O
	ATOM	4853	N	GLU	B	101	-3.784	-2.903	38.627	1.00	8.55	N
	ATOM	4855	CA	GLU	B	101	-3.315	-3.015	40.003	1.00	8.71	C
	ATOM	4857	CB	GLU	B	101	-4.160	-4.001	40.797	1.00	9.18	C
	ATOM	4860	CG	GLU	B	101	-3.907	-3.943	42.293	1.00	10.20	C

	ATOM	4863	CD	GLU	B	101	-5.020	-4.604	43.089	1.00	10.31	C
	ATOM	4864	OE1	GLU	B	101	-4.713	-5.401	43.998	1.00	12.33	O
	ATOM	4865	OE2	GLU	B	101	-6.210	-4.354	42.782	1.00	11.03	O
5	ATOM	4866	C	GLU	B	101	-1.858	-3.452	39.989	1.00	8.34	C
	ATOM	4867	O	GLU	B	101	-1.466	-4.253	39.161	1.00	9.30	O
	ATOM	4868	N	LEU	B	102	-1.073	-2.887	40.894	1.00	8.98	N
	ATOM	4870	CA	LEU	B	102	0.358	-3.079	40.934	1.00	8.90	C
	ATOM	4872	CB	LEU	B	102	1.068	-1.728	41.077	1.00	9.08	C
10	ATOM	4875	CG	LEU	B	102	0.752	-0.722	39.978	1.00	10.14	C
	ATOM	4877	CD1	LEU	B	102	1.517	0.561	40.225	1.00	10.89	C
	ATOM	4881	CD2	LEU	B	102	1.034	-1.294	38.585	1.00	10.99	C
	ATOM	4885	C	LEU	B	102	0.807	-3.976	42.080	1.00	9.48	C
	ATOM	4886	O	LEU	B	102	0.168	-4.061	43.133	1.00	10.17	O
	ATOM	4887	N	SER	B	103	1.969	-4.589	41.866	1.00	9.89	N
15	ATOM	4889	CA	SER	B	103	2.601	-5.483	42.828	1.00	10.79	C
	ATOM	4891	CB	SER	B	103	3.736	-6.273	42.146	1.00	11.99	C
	ATOM	4894	OG	SER	B	103	4.697	-5.398	41.584	1.00	15.05	O
	ATOM	4896	C	SER	B	103	3.183	-4.776	44.053	1.00	10.53	C
	ATOM	4897	O	SER	B	103	3.490	-5.433	45.047	1.00	11.56	O
20	ATOM	4898	N	GLU	B	104	3.367	-3.464	43.962	1.00	10.04	N
	ATOM	4900	CA	GLU	B	104	3.968	-2.672	45.021	1.00	10.08	C
	ATOM	4902	CB	GLU	B	104	5.443	-2.395	44.738	1.00	10.68	C
	ATOM	4905	CG	GLU	B	104	6.259	-3.644	44.449	1.00	11.66	C
	ATOM	4908	CD	GLU	B	104	7.723	-3.350	44.233	1.00	13.72	C
25	ATOM	4909	OE1	GLU	B	104	8.329	-2.659	45.084	1.00	14.76	O
	ATOM	4910	OE2	GLU	B	104	8.261	-3.802	43.208	1.00	19.30	O
	ATOM	4911	C	GLU	B	104	3.227	-1.351	45.093	1.00	9.84	C
	ATOM	4912	O	GLU	B	104	2.802	-0.809	44.065	1.00	10.32	O
	ATOM	4913	N	PRO	B	105	3.068	-0.805	46.291	1.00	9.86	N
30	ATOM	4914	CA	PRO	B	105	2.283	0.420	46.478	1.00	10.40	C
	ATOM	4916	CB	PRO	B	105	1.878	0.324	47.944	1.00	11.33	C
	ATOM	4919	CG	PRO	B	105	3.053	-0.322	48.587	1.00	11.44	C
	ATOM	4922	CD	PRO	B	105	3.557	-1.331	47.587	1.00	10.49	C
	ATOM	4925	C	PRO	B	105	3.075	1.696	46.191	1.00	9.58	C
35	ATOM	4926	O	PRO	B	105	3.227	2.576	47.035	1.00	10.04	O
	ATOM	4927	N	ILE	B	106	3.538	1.824	44.957	1.00	9.73	N
	ATOM	4929	CA	ILE	B	106	4.421	2.908	44.586	1.00	9.44	C
	ATOM	4931	CB	ILE	B	106	5.096	2.600	43.224	1.00	9.89	C
	ATOM	4933	CG1	ILE	B	106	6.252	3.566	42.933	1.00	10.25	C
40	ATOM	4936	CD1	ILE	B	106	7.381	3.581	43.970	1.00	11.38	C
	ATOM	4940	CG2	ILE	B	106	4.082	2.599	42.085	1.00	10.23	C
	ATOM	4944	C	ILE	B	106	3.729	4.271	44.620	1.00	9.14	C
	ATOM	4945	O	ILE	B	106	4.382	5.305	44.734	1.00	9.49	O
	ATOM	4946	N	GLY	B	107	2.407	4.287	44.541	1.00	9.13	N
45	ATOM	4948	CA	GLY	B	107	1.648	5.503	44.748	1.00	9.30	C
	ATOM	4951	C	GLY	B	107	1.833	6.128	46.117	1.00	9.86	C
	ATOM	4952	O	GLY	B	107	1.627	7.326	46.279	1.00	10.75	O
	ATOM	4953	N	ASN	B	108	2.228	5.339	47.110	1.00	10.06	N
	ATOM	4955	CA	ASN	B	108	2.578	5.915	48.400	1.00	11.08	C
50	ATOM	4957	CB	ASN	B	108	2.804	4.831	49.458	1.00	11.79	C
	ATOM	4960	CG	ASN	B	108	1.518	4.133	49.862	1.00	13.13	C
	ATOM	4961	OD1	ASN	B	108	0.433	4.675	49.715	1.00	15.54	O
	ATOM	4962	ND2	ASN	B	108	1.649	2.941	50.428	1.00	15.52	N
	ATOM	4965	C	ASN	B	108	3.799	6.809	48.340	1.00	11.38	C
55	ATOM	4966	O	ASN	B	108	3.968	7.676	49.192	1.00	13.40	O
	ATOM	4967	N	THR	B	109	4.644	6.606	47.335	1.00	10.92	N
	ATOM	4969	CA	THR	B	109	5.811	7.449	47.106	1.00	11.13	C
	ATOM	4971	CB	THR	B	109	6.961	6.584	46.594	1.00	11.44	C
	ATOM	4973	OG1	THR	B	109	7.329	5.636	47.604	1.00	13.07	O
60	ATOM	4975	CG2	THR	B	109	8.225	7.390	46.324	1.00	12.36	C
	ATOM	4979	C	THR	B	109	5.521	8.572	46.123	1.00	10.67	C
	ATOM	4980	O	THR	B	109	5.856	9.723	46.400	1.00	11.94	O
	ATOM	4981	N	VAL	B	110	4.931	8.247	44.975	1.00	9.89	N
	ATOM	4983	CA	VAL	B	110	4.771	9.245	43.921	1.00	9.99	C

	ATOM	4985	CB	VAL	B	110	4.883	8.652	42.504	1.00	9.69	C
	ATOM	4987	CG1	VAL	B	110	6.238	8.008	42.291	1.00	10.32	C
	ATOM	4991	CG2	VAL	B	110	3.749	7.687	42.194	1.00	9.18	C
5	ATOM	4995	C	VAL	B	110	3.512	10.093	44.054	1.00	10.21	C
	ATOM	4996	O	VAL	B	110	3.434	11.153	43.425	1.00	11.23	O
	ATOM	4997	N	GLY	B	111	2.543	9.644	44.840	1.00	10.22	N
	ATOM	4999	CA	GLY	B	111	1.265	10.314	44.904	1.00	10.24	C
	ATOM	5002	C	GLY	B	111	0.334	9.866	43.803	1.00	9.73	C
	ATOM	5003	O	GLY	B	111	0.623	8.938	43.039	1.00	10.12	O
10	ATOM	5004	N	TYR	B	112	-0.815	10.522	43.733	1.00	9.97	N
	ATOM	5006	CA	TYR	B	112	-1.832	10.140	42.768	1.00	9.95	C
	ATOM	5008	CB	BTYR	B	112	-2.648	8.897	43.221	0.35	10.43	C
	ATOM	5009	CB	ATYR	B	112	-2.598	8.884	43.221	0.65	10.39	C
	ATOM	5014	CG	BTYR	B	112	-2.791	8.641	44.714	0.35	11.58	C
15	ATOM	5015	CG	ATYR	B	112	-3.133	8.921	44.615	0.65	11.41	C
	ATOM	5016	CD1	BTYR	B	112	-1.797	7.973	45.428	0.35	12.47	C
	ATOM	5017	CD1	ATYR	B	112	-2.406	8.376	45.672	0.65	13.18	C
	ATOM	5020	CE1	BTYR	B	112	-1.935	7.713	46.789	0.35	13.56	C
	ATOM	5021	CE1	ATYR	B	112	-2.905	8.381	46.970	0.65	15.52	C
20	ATOM	5024	CZ	BTYR	B	112	-3.085	8.100	47.449	0.35	14.96	C
	ATOM	5025	CZ	ATYR	B	112	-4.144	8.931	47.209	0.65	16.29	C
	ATOM	5026	OH	BTYR	B	112	-3.215	7.838	48.796	0.35	16.64	O
	ATOM	5027	OH	ATYR	B	112	-4.641	8.940	48.492	0.65	18.51	O
	ATOM	5030	CE2	BTYR	B	112	-4.097	8.743	46.766	0.35	14.31	C
25	ATOM	5031	CE2	ATYR	B	112	-4.894	9.467	46.174	0.65	14.98	C
	ATOM	5034	CD2	BTYR	B	112	-3.951	9.007	45.400	0.35	12.89	C
	ATOM	5035	CD2	ATYR	B	112	-4.382	9.459	44.880	0.65	13.02	C
	ATOM	5038	C	TYR	B	112	-2.745	11.327	42.440	1.00	9.75	C
	ATOM	5039	O	TYR	B	112	-2.730	12.363	43.110	1.00	10.71	O
30	ATOM	5040	N	PHE	B	113	-3.495	11.159	41.355	1.00	9.55	N
	ATOM	5042	CA	PHE	B	113	-4.382	12.182	40.822	1.00	9.90	C
	ATOM	5044	CB	PHE	B	113	-4.592	11.952	39.321	1.00	9.79	C
	ATOM	5047	CG	PHE	B	113	-3.437	12.384	38.452	1.00	8.75	C
	ATOM	5048	CD1	PHE	B	113	-3.520	13.562	37.714	1.00	9.30	C
35	ATOM	5050	CE1	PHE	B	113	-2.467	13.968	36.912	1.00	10.00	C
	ATOM	5052	CZ	PHE	B	113	-1.321	13.222	36.851	1.00	8.88	C
	ATOM	5054	CE2	PHE	B	113	-1.220	12.060	37.566	1.00	8.70	C
	ATOM	5056	CD2	PHE	B	113	-2.271	11.633	38.372	1.00	8.97	C
	ATOM	5058	C	PHE	B	113	-5.772	12.106	41.441	1.00	10.47	C
40	ATOM	5059	O	PHE	B	113	-6.262	11.022	41.775	1.00	11.47	O
	ATOM	5060	N	GLY	B	114	-6.409	13.267	41.550	1.00	10.79	N
	ATOM	5062	CA	GLY	B	114	-7.854	13.333	41.663	1.00	11.39	C
	ATOM	5065	C	GLY	B	114	-8.481	13.125	40.293	1.00	10.67	C
	ATOM	5066	O	GLY	B	114	-7.801	13.207	39.265	1.00	10.78	O
45	ATOM	5067	N	TYR	B	115	-9.781	12.875	40.278	1.00	10.57	N
	ATOM	5069	CA	TYR	B	115	-10.524	12.763	39.030	1.00	10.75	C
	ATOM	5071	CB	TYR	B	115	-10.346	11.382	38.379	1.00	10.71	C
	ATOM	5074	CG	TYR	B	115	-10.685	10.219	39.275	1.00	10.79	C
	ATOM	5075	CD1	TYR	B	115	-11.988	9.716	39.338	1.00	10.63	C
50	ATOM	5077	CE1	TYR	B	115	-12.311	8.658	40.183	1.00	10.32	C
	ATOM	5079	CZ	TYR	B	115	-11.313	8.093	40.968	1.00	10.91	C
	ATOM	5080	OH	TYR	B	115	-11.581	7.056	41.831	1.00	12.42	O
	ATOM	5082	CE2	TYR	B	115	-10.021	8.585	40.921	1.00	11.68	C
	ATOM	5084	CD2	TYR	B	115	-9.715	9.638	40.074	1.00	11.40	C
55	ATOM	5086	C	TYR	B	115	-11.983	13.069	39.319	1.00	10.14	C
	ATOM	5087	O	TYR	B	115	-12.466	12.866	40.448	1.00	11.11	O
	ATOM	5088	N	SER	B	116	-12.696	13.556	38.315	1.00	10.15	N
	ATOM	5090	CA	SER	B	116	-14.058	14.032	38.525	1.00	11.05	C
	ATOM	5092	CB	SER	B	116	-14.047	15.464	39.061	1.00	12.22	C
60	ATOM	5095	OG	SER	B	116	-15.261	15.741	39.743	1.00	15.63	O
	ATOM	5097	C	SER	B	116	-14.881	13.963	37.258	1.00	10.80	C
	ATOM	5098	O	SER	B	116	-14.333	13.881	36.155	1.00	11.34	O
	ATOM	5099	N	TYR	B	117	-16.198	13.964	37.448	1.00	11.27	N
	ATOM	5101	CA	TYR	B	117	-17.167	14.054	36.366	1.00	11.28	C

5	ATOM	5103	CB	TYR	B	117	-18.098	12.834	36.354	1.00	11.24	C
	ATOM	5106	CG	TYR	B	117	-19.039	12.746	37.533	1.00	11.74	C
	ATOM	5107	CD1	TYR	B	117	-20.343	13.212	37.431	1.00	13.27	C
	ATOM	5109	CE1	TYR	B	117	-21.221	13.145	38.509	1.00	15.32	C
	ATOM	5111	CZ	TYR	B	117	-20.788	12.637	39.708	1.00	15.51	C
	ATOM	5112	OH	TYR	B	117	-21.659	12.576	40.774	1.00	18.16	O
	ATOM	5114	CE2	TYR	B	117	-19.494	12.174	39.841	1.00	15.41	C
	ATOM	5116	CD2	TYR	B	117	-18.626	12.229	38.758	1.00	13.41	C
10	ATOM	5118	C	TYR	B	117	-17.976	15.325	36.528	1.00	12.11	C
	ATOM	5119	O	TYR	B	117	-18.090	15.880	37.624	1.00	13.00	O
	ATOM	5120	N	THR	B	118	-18.546	15.790	35.430	1.00	12.32	N
	ATOM	5122	CA	THR	B	118	-19.471	16.915	35.476	1.00	13.14	C
15	ATOM	5124	CB	BTHR	B	118	-18.839	18.242	34.989	0.35	13.58	C
	ATOM	5125	CB	ATHR	B	118	-18.853	18.174	34.815	0.65	13.85	C
	ATOM	5128	OG1BTHR	B	118	-17.607	18.487	35.674	0.35	14.98	O	
	ATOM	5129	OG1ATHR	B	118	-18.864	18.025	33.391	0.65	12.42	O	
	ATOM	5132	CG2BTHR	B	118	-19.688	19.435	35.421	0.35	13.29	C	
	ATOM	5133	CG2ATHR	B	118	-17.368	18.339	35.127	0.65	14.99	C	
20	ATOM	5140	C	THR	B	118	-20.722	16.573	34.714	1.00	13.65	C
	ATOM	5141	O	THR	B	118	-20.751	15.691	33.870	1.00	14.93	O
	ATOM	5142	N	THR	B	119	-21.782	17.313	35.018	1.00	14.79	N
	ATOM	5144	CA	THR	B	119	-23.087	17.127	34.387	1.00	16.50	C
25	ATOM	5146	CB	THR	B	119	-24.192	17.090	35.473	1.00	17.34	C
	ATOM	5148	OG1	THR	B	119	-24.184	18.319	36.209	1.00	19.76	O
	ATOM	5150	CG2	THR	B	119	-23.906	16.005	36.521	1.00	18.20	C
	ATOM	5154	C	THR	B	119	-23.412	18.233	33.389	1.00	16.89	C
	ATOM	5155	O	THR	B	119	-24.581	18.446	33.065	1.00	18.39	O
30	ATOM	5156	N	SER	B	120	-22.392	18.945	32.932	1.00	15.85	N
	ATOM	5158	CA	SER	B	120	-22.568	20.023	31.976	1.00	15.52	C
	ATOM	5160	CB	SER	B	120	-22.688	21.348	32.714	1.00	17.29	C
	ATOM	5163	OG	SER	B	120	-21.566	21.555	33.538	1.00	19.16	O
	ATOM	5165	C	SER	B	120	-21.385	20.044	31.015	1.00	13.69	C
	ATOM	5166	O	SER	B	120	-20.433	19.256	31.151	1.00	13.49	O
35	ATOM	5167	N	SER	B	121	-21.450	20.938	30.037	1.00	13.50	N
	ATOM	5169	CA	SER	B	121	-20.440	20.977	28.999	1.00	13.20	C
	ATOM	5171	CB	SER	B	121	-20.823	22.004	27.943	1.00	13.58	C
	ATOM	5174	OG	SER	B	121	-19.822	22.072	26.951	1.00	14.79	O
40	ATOM	5176	C	SER	B	121	-19.065	21.321	29.561	1.00	12.29	C
	ATOM	5177	O	SER	B	121	-18.936	22.162	30.445	1.00	13.82	O
	ATOM	5178	N	LEU	B	122	-18.034	20.659	29.042	1.00	11.06	N
	ATOM	5180	CA	LEU	B	122	-16.653	20.994	29.362	1.00	10.63	C
	ATOM	5182	CB	LEU	B	122	-15.870	19.715	29.679	1.00	10.33	C
45	ATOM	5185	CG	LEU	B	122	-16.152	19.154	31.076	1.00	11.02	C
	ATOM	5187	CD1	LEU	B	122	-15.645	17.729	31.205	1.00	11.81	C
	ATOM	5191	CD2	LEU	B	122	-15.557	20.038	32.139	1.00	12.56	C
	ATOM	5195	C	LEU	B	122	-15.968	21.775	28.238	1.00	10.13	C
	ATOM	5196	O	LEU	B	122	-14.775	22.042	28.324	1.00	10.57	O
50	ATOM	5197	N	VAL	B	123	-16.708	22.183	27.209	1.00	10.64	N
	ATOM	5199	CA	VAL	B	123	-16.101	22.935	26.115	1.00	10.83	C
	ATOM	5201	CB	VAL	B	123	-17.123	23.312	25.017	1.00	11.49	C
	ATOM	5203	CG1	VAL	B	123	-16.511	24.290	24.006	1.00	12.60	C
	ATOM	5207	CG2	VAL	B	123	-17.603	22.060	24.288	1.00	12.49	C
55	ATOM	5211	C	VAL	B	123	-15.439	24.192	26.669	1.00	10.32	C
	ATOM	5212	O	VAL	B	123	-16.057	24.936	27.431	1.00	11.74	O
	ATOM	5213	N	GLY	B	124	-14.189	24.416	26.283	1.00	10.03	N
	ATOM	5215	CA	GLY	B	124	-13.431	25.575	26.714	1.00	10.35	C
	ATOM	5218	C	GLY	B	124	-12.591	25.362	27.954	1.00	9.90	C
60	ATOM	5219	O	GLY	B	124	-11.707	26.170	28.220	1.00	11.28	O
	ATOM	5220	N	THR	B	125	-12.851	24.311	28.726	1.00	9.67	N
	ATOM	5222	CA	THR	B	125	-12.062	24.049	29.919	1.00	9.49	C
	ATOM	5224	CB	THR	B	125	-12.709	22.900	30.695	1.00	10.53	C
	ATOM	5226	OG1	THR	B	125	-13.998	23.310	31.178	1.00	13.20	O
	ATOM	5228	CG2	THR	B	125	-11.907	22.498	31.922	1.00	10.73	C
	ATOM	5232	C	THR	B	125	-10.635	23.689	29.511	1.00	8.89	C

	ATOM	5233	O	THR	B	125	-10.437	22.872	28.619	1.00	9.49	O
	ATOM	5234	N	THR	B	126	-9.646	24.285	30.170	1.00	9.07	N
	ATOM	5236	CA	THR	B	126	-8.254	23.992	29.867	1.00	9.38	C
5	ATOM	5238	CB	THR	B	126	-7.368	25.212	30.064	1.00	10.46	C
	ATOM	5240	OG1	THR	B	126	-7.532	25.706	31.393	1.00	12.73	O
	ATOM	5242	CG2	THR	B	126	-7.790	26.346	29.130	1.00	11.44	C
	ATOM	5246	C	THR	B	126	-7.731	22.819	30.679	1.00	8.61	C
	ATOM	5247	O	THR	B	126	-8.035	22.654	31.874	1.00	9.84	O
	ATOM	5248	N	VAL	B	127	-6.951	21.996	29.987	1.00	8.18	N
10	ATOM	5250	CA	VAL	B	127	-6.403	20.764	30.520	1.00	8.03	C
	ATOM	5252	CB	VAL	B	127	-7.290	19.529	30.187	1.00	8.26	C
	ATOM	5254	CG1	VAL	B	127	-8.635	19.599	30.912	1.00	9.66	C
	ATOM	5258	CG2	VAL	B	127	-7.486	19.389	28.694	1.00	9.04	C
	ATOM	5262	C	VAL	B	127	-5.001	20.543	29.961	1.00	7.96	C
15	ATOM	5263	O	VAL	B	127	-4.625	21.117	28.935	1.00	9.12	O
	ATOM	5264	N	THR	B	128	-4.259	19.675	30.630	1.00	7.81	N
	ATOM	5266	CA	THR	B	128	-2.953	19.208	30.209	1.00	7.75	C
	ATOM	5268	CB	THR	B	128	-1.953	19.374	31.362	1.00	8.07	C
	ATOM	5270	OG1	THR	B	128	-1.843	20.762	31.705	1.00	9.24	O
20	ATOM	5272	CG2	THR	B	128	-0.549	18.864	31.006	1.00	8.88	C
	ATOM	5276	C	THR	B	128	-3.052	17.735	29.857	1.00	7.12	C
	ATOM	5277	O	THR	B	128	-3.715	16.967	30.556	1.00	7.80	O
	ATOM	5278	N	ILE	B	129	-2.385	17.340	28.775	1.00	6.77	N
	ATOM	5280	CA	ILE	B	129	-2.233	15.940	28.421	1.00	6.79	C
25	ATOM	5282	CB	ILE	B	129	-2.874	15.613	27.062	1.00	7.12	C
	ATOM	5284	CG1	ILE	B	129	-4.328	16.098	27.046	1.00	7.95	C
	ATOM	5287	CD1	ILE	B	129	-5.076	15.825	25.764	1.00	8.74	C
	ATOM	5291	CG2	ILE	B	129	-2.766	14.141	26.789	1.00	8.17	C
	ATOM	5295	C	ILE	B	129	-0.739	15.639	28.412	1.00	6.77	C
30	ATOM	5296	O	ILE	B	129	0.001	16.217	27.603	1.00	7.27	O
	ATOM	5297	N	SER	B	130	-0.298	14.761	29.305	1.00	6.76	N
	ATOM	5299	CA	SER	B	130	1.112	14.417	29.438	1.00	6.68	C
	ATOM	5301	CB	SER	B	130	1.694	15.022	30.710	1.00	7.29	C
	ATOM	5304	OG	SER	B	130	3.097	14.906	30.734	1.00	8.01	O
35	ATOM	5306	C	SER	B	130	1.250	12.911	29.453	1.00	6.73	C
	ATOM	5307	O	SER	B	130	0.517	12.224	30.158	1.00	6.79	O
	ATOM	5308	N	GLY	B	131	2.187	12.390	28.665	1.00	6.62	N
	ATOM	5310	CA	GLY	B	131	2.425	10.958	28.637	1.00	6.73	C
	ATOM	5313	C	GLY	B	131	3.640	10.604	27.804	1.00	6.56	C
40	ATOM	5314	O	GLY	B	131	4.554	11.409	27.661	1.00	7.16	O
	ATOM	5315	N	TYR	B	132	3.652	9.381	27.288	1.00	6.84	N
	ATOM	5317	CA	TYR	B	132	4.858	8.737	26.740	1.00	7.12	C
	ATOM	5319	CB	TYR	B	132	5.165	7.463	27.555	1.00	7.14	C
	ATOM	5322	CG	TYR	B	132	5.728	7.832	28.917	1.00	7.14	C
45	ATOM	5323	CD1	TYR	B	132	7.087	8.103	29.060	1.00	7.37	C
	ATOM	5325	CE1	TYR	B	132	7.614	8.520	30.265	1.00	7.89	C
	ATOM	5327	CZ	TYR	B	132	6.781	8.669	31.364	1.00	7.57	C
	ATOM	5328	OH	TYR	B	132	7.269	9.112	32.573	1.00	8.04	O
	ATOM	5330	CE2	TYR	B	132	5.438	8.389	31.262	1.00	7.67	C
50	ATOM	5332	CD2	TYR	B	132	4.908	7.980	30.035	1.00	7.29	C
	ATOM	5334	C	TYR	B	132	4.676	8.424	25.250	1.00	6.94	C
	ATOM	5335	O	TYR	B	132	4.361	7.295	24.880	1.00	8.05	O
	ATOM	5336	N	PRO	B	133	4.874	9.411	24.378	1.00	7.25	N
	ATOM	5337	CA	PRO	B	133	4.670	9.185	22.944	1.00	7.42	C
55	ATOM	5339	CB	PRO	B	133	4.628	10.594	22.368	1.00	8.13	C
	ATOM	5342	CG	PRO	B	133	5.503	11.387	23.285	1.00	8.21	C
	ATOM	5345	CD	PRO	B	133	5.210	10.826	24.655	1.00	7.47	C
	ATOM	5348	C	PRO	B	133	5.786	8.400	22.267	1.00	7.76	C
	ATOM	5349	O	PRO	B	133	6.974	8.597	22.533	1.00	8.78	O
60	ATOM	5350	N	GLY	B	134	5.389	7.581	21.300	1.00	8.21	N
	ATOM	5352	CA	GLY	B	134	6.306	6.749	20.548	1.00	9.26	C
	ATOM	5355	C	GLY	B	134	7.046	7.440	19.418	1.00	9.69	C
	ATOM	5356	O	GLY	B	134	7.926	6.828	18.819	1.00	12.46	O
	ATOM	5357	N	ASP	B	135	6.718	8.697	19.134	1.00	8.82	N

	ATOM	5359	CA	ASP	B	135	7.459	9.489	18.154	1.00	9.23	C
	ATOM	5361	CB	ASP	B	135	6.533	10.305	17.243	1.00	8.88	C
	ATOM	5364	CG	ASP	B	135	5.732	11.364	17.966	1.00	8.72	C
5	ATOM	5365	OD1	ASP	B	135	5.506	11.238	19.200	1.00	8.57	O
	ATOM	5366	OD2	ASP	B	135	5.290	12.341	17.292	1.00	9.32	O
	ATOM	5367	C	ASP	B	135	8.523	10.368	18.796	1.00	9.65	C
	ATOM	5368	O	ASP	B	135	9.102	11.216	18.121	1.00	11.42	O
	ATOM	5369	N	LYS	B	136	8.768	10.161	20.088	1.00	9.77	N
	ATOM	5371	CA	LYS	B	136	9.873	10.781	20.812	1.00	10.15	C
10	ATOM	5373	CB	LYS	B	136	9.349	11.647	21.958	1.00	9.99	C
	ATOM	5376	CG	LYS	B	136	8.378	12.734	21.523	1.00	10.04	C
	ATOM	5379	CD	LYS	B	136	9.008	13.792	20.637	1.00	11.86	C
	ATOM	5382	CE	LYS	B	136	8.014	14.925	20.392	1.00	13.11	C
	ATOM	5385	NZ	LYS	B	136	8.453	15.910	19.384	1.00	15.13	N
15	ATOM	5389	C	LYS	B	136	10.756	9.670	21.376	1.00	10.43	C
	ATOM	5390	O	LYS	B	136	10.432	8.491	21.280	1.00	11.37	O
	ATOM	5391	N	THR	B	137	11.881	10.042	21.976	1.00	11.03	N
	ATOM	5393	CA	THR	B	137	12.777	9.068	22.582	1.00	11.49	C
	ATOM	5395	CB	THR	B	137	13.887	9.813	23.343	1.00	12.46	C
20	ATOM	5397	OG1	THR	B	137	14.687	10.558	22.415	1.00	14.16	O
	ATOM	5399	CG2	THR	B	137	14.865	8.837	24.040	1.00	13.85	C
	ATOM	5403	C	THR	B	137	12.010	8.169	23.536	1.00	10.34	C
	ATOM	5404	O	THR	B	137	11.257	8.654	24.378	1.00	9.84	O
	ATOM	5405	N	ALA	B	138	12.240	6.868	23.428	1.00	10.82	N
25	ATOM	5407	CA	ALA	B	138	11.524	5.900	24.232	1.00	10.91	C
	ATOM	5409	CB	ALA	B	138	12.055	4.511	23.995	1.00	11.85	C
	ATOM	5413	C	ALA	B	138	11.631	6.256	25.702	1.00	10.45	C
	ATOM	5414	O	ALA	B	138	12.694	6.552	26.218	1.00	11.32	O
	ATOM	5415	N	GLY	B	139	10.503	6.194	26.378	1.00	10.16	N
30	ATOM	5417	CA	GLY	B	139	10.468	6.419	27.800	1.00	9.75	C
	ATOM	5420	C	GLY	B	139	10.535	7.861	28.250	1.00	8.52	C
	ATOM	5421	O	GLY	B	139	10.669	8.089	29.441	1.00	9.08	O
	ATOM	5422	N	THR	B	140	10.421	8.829	27.340	1.00	8.37	N
	ATOM	5424	CA	THR	B	140	10.416	10.238	27.729	1.00	8.32	C
35	ATOM	5426	CB	THR	B	140	11.318	11.119	26.843	1.00	9.11	C
	ATOM	5428	OG1	THR	B	140	10.877	11.095	25.476	1.00	9.56	O
	ATOM	5430	CG2	THR	B	140	12.768	10.611	26.900	1.00	10.40	C
	ATOM	5434	C	THR	B	140	8.987	10.774	27.783	1.00	7.64	C
	ATOM	5435	O	THR	B	140	8.120	10.378	26.991	1.00	8.11	O
40	ATOM	5436	N	GLN	B	141	8.736	11.644	28.753	1.00	7.31	N
	ATOM	5438	CA	GLN	B	141	7.405	12.176	28.997	1.00	7.20	C
	ATOM	5440	CB	GLN	B	141	7.108	12.226	30.513	1.00	7.37	C
	ATOM	5443	CG	GLN	B	141	5.617	12.242	30.802	1.00	7.74	C
	ATOM	5446	CD	GLN	B	141	5.238	12.460	32.256	1.00	7.09	C
45	ATOM	5447	OE1	GLN	B	141	4.394	13.318	32.560	1.00	8.12	O
	ATOM	5448	NE2	GLN	B	141	5.812	11.669	33.171	1.00	7.80	N
	ATOM	5451	C	GLN	B	141	7.284	13.551	28.353	1.00	7.04	C
	ATOM	5452	O	GLN	B	141	8.177	14.384	28.523	1.00	7.62	O
	ATOM	5453	N	TRP	B	142	6.180	13.771	27.652	1.00	7.07	N
50	ATOM	5455	CA	TRP	B	142	5.912	14.982	26.902	1.00	7.12	C
	ATOM	5457	CB	TRP	B	142	6.063	14.717	25.387	1.00	7.52	C
	ATOM	5460	CG	TRP	B	142	7.481	14.449	24.987	1.00	7.95	C
	ATOM	5461	CD1	TRP	B	142	8.205	13.342	25.263	1.00	7.71	C
	ATOM	5463	NE1	TRP	B	142	9.486	13.476	24.796	1.00	8.83	N
55	ATOM	5465	CE2	TRP	B	142	9.612	14.702	24.206	1.00	8.82	C
	ATOM	5466	CD2	TRP	B	142	8.363	15.342	24.303	1.00	8.41	C
	ATOM	5467	CE3	TRP	B	142	8.227	16.617	23.758	1.00	9.13	C
	ATOM	5469	CZ3	TRP	B	142	9.321	17.200	23.122	1.00	9.87	C
	ATOM	5471	CH2	TRP	B	142	10.538	16.541	23.048	1.00	10.47	C
60	ATOM	5473	CZ2	TRP	B	142	10.703	15.288	23.568	1.00	10.08	C
	ATOM	5475	C	TRP	B	142	4.492	15.441	27.166	1.00	6.82	C
	ATOM	5476	O	TRP	B	142	3.594	14.623	27.352	1.00	7.54	O
	ATOM	5477	N	GLN	B	143	4.282	16.757	27.146	1.00	6.91	N
	ATOM	5479	CA	GLN	B	143	2.998	17.337	27.499	1.00	6.91	C

	ATOM	5481	CB	GLN	B	143	3.012	17.856	28.938	1.00	7.31	C
	ATOM	5484	CG	GLN	B	143	3.928	19.058	29.162	1.00	8.18	C
	ATOM	5487	CD	GLN	B	143	3.867	19.564	30.570	1.00	8.88	C
5	ATOM	5488	OE1	GLN	B	143	2.792	19.555	31.173	1.00	10.18	O
	ATOM	5489	NE2	GLN	B	143	4.988	20.039	31.087	1.00	10.86	N
	ATOM	5492	C	GLN	B	143	2.599	18.460	26.561	1.00	7.08	C
	ATOM	5493	O	GLN	B	143	3.427	19.104	25.928	1.00	7.42	O
	ATOM	5494	N	HIS	B	144	1.296	18.711	26.521	1.00	7.02	N
	ATOM	5496	CA	HIS	B	144	0.706	19.829	25.804	1.00	7.09	C
10	ATOM	5498	CB	HIS	B	144	0.457	19.463	24.342	1.00	7.49	C
	ATOM	5501	CG	HIS	B	144	0.061	20.617	23.491	1.00	7.31	C
	ATOM	5502	ND1	HIS	B	144	-0.682	20.454	22.350	1.00	8.42	N
	ATOM	5504	CE1	HIS	B	144	-0.861	21.643	21.800	1.00	8.61	C
	ATOM	5506	NE2	HIS	B	144	-0.286	22.564	22.557	1.00	8.06	N
15	ATOM	5508	CD2	HIS	B	144	0.319	21.942	23.610	1.00	8.23	C
	ATOM	5510	C	HIS	B	144	-0.604	20.173	26.496	1.00	6.98	C
	ATOM	5511	O	HIS	B	144	-1.362	19.276	26.890	1.00	8.41	O
	ATOM	5512	N	SER	B	145	-0.878	21.463	26.640	1.00	7.81	N
20	ATOM	5514	CA	SER	B	145	-2.093	21.946	27.290	1.00	7.65	C
	ATOM	5516	CB	SER	B	145	-1.755	22.780	28.522	1.00	8.72	C
	ATOM	5519	OG	SER	B	145	-1.027	22.028	29.472	1.00	9.99	O
	ATOM	5521	C	SER	B	145	-2.927	22.764	26.315	1.00	7.50	C
	ATOM	5522	O	SER	B	145	-2.406	23.304	25.338	1.00	8.51	O
25	ATOM	5523	N	GLY	B	146	-4.218	22.863	26.598	1.00	7.67	N
	ATOM	5525	CA	GLY	B	146	-5.132	23.637	25.793	1.00	7.84	C
	ATOM	5528	C	GLY	B	146	-6.563	23.318	26.170	1.00	7.50	C
	ATOM	5529	O	GLY	B	146	-6.830	22.629	27.148	1.00	8.13	O
	ATOM	5530	N	PRO	B	147	-7.503	23.835	25.402	1.00	7.99	N
30	ATOM	5531	CA	PRO	B	147	-8.924	23.707	25.733	1.00	8.42	C
	ATOM	5533	CB	PRO	B	147	-9.524	24.935	25.053	1.00	8.99	C
	ATOM	5536	CG	PRO	B	147	-8.686	25.096	23.804	1.00	9.17	C
	ATOM	5539	CD	PRO	B	147	-7.290	24.658	24.195	1.00	8.66	C
	ATOM	5542	C	PRO	B	147	-9.581	22.445	25.182	1.00	8.24	C
	ATOM	5543	O	PRO	B	147	-9.216	21.930	24.128	1.00	8.60	O
35	ATOM	5544	N	ILE	B	148	-10.613	21.991	25.892	1.00	8.18	N
	ATOM	5546	CA	ILE	B	148	-11.532	21.003	25.349	1.00	8.21	C
	ATOM	5548	CB	ILE	B	148	-12.458	20.481	26.455	1.00	8.12	C
	ATOM	5550	CG1	ILE	B	148	-11.654	19.795	27.570	1.00	9.56	C
40	ATOM	5553	CD1	ILE	B	148	-10.843	18.627	27.145	1.00	10.59	C
	ATOM	5557	CG2	ILE	B	148	-13.529	19.585	25.887	1.00	8.52	C
	ATOM	5561	C	ILE	B	148	-12.338	21.662	24.222	1.00	8.21	C
	ATOM	5562	O	ILE	B	148	-12.939	22.728	24.410	1.00	9.55	O
	ATOM	5563	N	ALA	B	149	-12.348	21.019	23.064	1.00	8.67	N
45	ATOM	5565	CA	ALA	B	149	-13.055	21.532	21.896	1.00	9.10	C
	ATOM	5567	CB	ALA	B	149	-12.286	21.197	20.632	1.00	9.84	C
	ATOM	5571	C	ALA	B	149	-14.476	21.013	21.771	1.00	9.41	C
	ATOM	5572	O	ALA	B	149	-15.352	21.743	21.301	1.00	10.63	O
	ATOM	5573	N	ILE	B	150	-14.684	19.747	22.136	1.00	9.20	N
50	ATOM	5575	CA	ILE	B	150	-15.983	19.098	22.036	1.00	10.09	C
	ATOM	5577	CB	ILE	B	150	-16.093	18.145	20.814	1.00	10.95	C
	ATOM	5579	CG1	ILE	B	150	-15.739	18.858	19.510	1.00	10.69	C
	ATOM	5582	CD1	ILE	B	150	-15.768	17.974	18.271	1.00	11.51	C
	ATOM	5586	CG2	ILE	B	150	-17.497	17.568	20.704	1.00	13.68	C
55	ATOM	5590	C	ILE	B	150	-16.183	18.306	23.320	1.00	9.44	C
	ATOM	5591	O	ILE	B	150	-15.291	17.594	23.769	1.00	8.76	O
	ATOM	5592	N	SER	B	151	-17.372	18.427	23.889	1.00	10.89	N
	ATOM	5594	CA	SER	B	151	-17.765	17.741	25.101	1.00	11.77	C
	ATOM	5596	CB	SER	B	151	-18.167	18.810	26.136	1.00	12.97	C
60	ATOM	5599	OG	SER	B	151	-18.512	18.242	27.381	1.00	15.09	O
	ATOM	5601	C	SER	B	151	-18.973	16.866	24.767	1.00	11.59	C
	ATOM	5602	O	SER	B	151	-20.087	17.374	24.707	1.00	14.34	O
	ATOM	5603	N	GLU	B	152	-18.761	15.581	24.505	1.00	11.06	N
	ATOM	5605	CA	GLU	B	152	-19.854	14.663	24.213	1.00	10.89	C
	ATOM	5607	CB	BGLU	B	152	-19.631	13.897	22.888	0.35	11.93	C

	ATOM	5608	CB	AGLU	B	152	-19.474	13.796	23.024	0.65	12.76	C
	ATOM	5613	CG	BGLU	B	152	-20.041	14.715	21.652	0.35	12.04	C
	ATOM	5614	CG	AGLU	B	152	-18.748	14.582	21.953	0.65	14.68	C
	ATOM	5619	CD	BGLU	B	152	-20.198	13.882	20.388	0.35	13.76	C
5	ATOM	5620	CD	AGLU	B	152	-18.197	13.685	20.889	0.65	17.47	C
	ATOM	5621	OE1	BGLU	B	152	-21.155	14.126	19.613	0.35	15.60	O
	ATOM	5622	OE1	AGLU	B	152	-18.974	13.391	19.960	0.65	19.10	O
	ATOM	5623	OE2	BGLU	B	152	-19.369	12.976	20.169	0.35	15.01	O
	ATOM	5624	OE2	AGLU	B	152	-17.012	13.276	20.998	0.65	18.63	O
10	ATOM	5625	C	GLU	B	152	-20.076	13.771	25.417	1.00	10.28	C
	ATOM	5626	O	GLU	B	152	-19.376	13.873	26.426	1.00	11.20	O
	ATOM	5627	N	THR	B	153	-21.057	12.893	25.338	1.00	9.81	N
	ATOM	5629	CA	THR	B	153	-21.430	12.101	26.492	1.00	10.13	C
	ATOM	5631	CB	THR	B	153	-22.622	11.232	26.129	1.00	10.71	C
15	ATOM	5633	OG1	THR	B	153	-23.706	12.086	25.751	1.00	12.66	O
	ATOM	5635	CG2	THR	B	153	-23.106	10.417	27.332	1.00	11.52	C
	ATOM	5639	C	THR	B	153	-20.286	11.246	27.012	1.00	9.76	C
	ATOM	5640	O	THR	B	153	-20.065	11.197	28.214	1.00	10.41	O
	ATOM	5641	N	TYR	B	154	-19.588	10.574	26.108	1.00	9.41	N
20	ATOM	5643	CA	TYR	B	154	-18.588	9.578	26.489	1.00	9.25	C
	ATOM	5645	CB	TYR	B	154	-18.890	8.217	25.847	1.00	9.78	C
	ATOM	5648	CG	TYR	B	154	-20.239	7.693	26.220	1.00	10.14	C
	ATOM	5649	CD1	TYR	B	154	-20.470	7.150	27.475	1.00	10.50	C
	ATOM	5651	CE1	TYR	B	154	-21.712	6.673	27.838	1.00	10.83	C
25	ATOM	5653	CZ	TYR	B	154	-22.755	6.754	26.930	1.00	11.01	C
	ATOM	5654	OH	TYR	B	154	-24.016	6.299	27.224	1.00	12.69	O
	ATOM	5656	CE2	TYR	B	154	-22.535	7.282	25.686	1.00	11.61	C
	ATOM	5658	CD2	TYR	B	154	-21.293	7.762	25.344	1.00	11.24	C
	ATOM	5660	C	TYR	B	154	-17.172	9.972	26.116	1.00	8.89	C
30	ATOM	5661	O	TYR	B	154	-16.233	9.282	26.532	1.00	9.04	O
	ATOM	5662	N	LYS	B	155	-17.001	11.045	25.351	1.00	9.30	N
	ATOM	5664	CA	LYS	B	155	-15.694	11.445	24.860	1.00	9.53	C
	ATOM	5666	CB	BLYS	B	155	-15.479	11.009	23.401	0.35	10.42	C
	ATOM	5667	CB	ALYS	B	155	-15.521	11.057	23.393	0.65	10.60	C
35	ATOM	5672	CG	BLYS	B	155	-15.714	9.526	23.080	0.35	11.44	C
	ATOM	5673	CG	ALYS	B	155	-15.446	9.579	23.102	0.65	11.66	C
	ATOM	5678	CD	BLYS	B	155	-14.796	8.573	23.861	0.35	11.42	C
	ATOM	5679	CD	ALYS	B	155	-14.096	8.991	23.466	0.65	9.69	C
	ATOM	5684	CE	BLYS	B	155	-13.424	8.327	23.221	0.35	11.05	C
40	ATOM	5685	CE	ALYS	B	155	-14.129	7.489	23.408	0.65	12.11	C
	ATOM	5690	NZ	BLYS	B	155	-12.677	7.235	23.943	0.35	10.75	N
	ATOM	5691	NZ	ALYS	B	155	-12.784	6.834	23.478	0.65	10.75	N
	ATOM	5698	C	LYS	B	155	-15.565	12.944	24.954	1.00	9.70	C
	ATOM	5699	O	LYS	B	155	-16.531	13.686	24.765	1.00	11.39	O
45	ATOM	5700	N	LEU	B	156	-14.365	13.388	25.280	1.00	8.75	N
	ATOM	5702	CA	LEU	B	156	-13.957	14.757	25.042	1.00	8.77	C
	ATOM	5704	CB	LEU	B	156	-13.188	15.313	26.238	1.00	9.34	C
	ATOM	5707	CG	LEU	B	156	-13.899	15.230	27.589	1.00	9.50	C
	ATOM	5709	CD1	LEU	B	156	-13.075	15.921	28.641	1.00	10.29	C
50	ATOM	5713	CD2	LEU	B	156	-15.313	15.818	27.545	1.00	10.26	C
	ATOM	5717	C	LEU	B	156	-13.063	14.781	23.817	1.00	8.24	C
	ATOM	5718	O	LEU	B	156	-12.322	13.817	23.555	1.00	9.48	O
	ATOM	5719	N	GLN	B	157	-13.115	15.863	23.049	1.00	7.55	N
	ATOM	5721	CA	GLN	B	157	-12.210	16.040	21.931	1.00	7.62	C
55	ATOM	5723	CB	GLN	B	157	-12.926	15.991	20.589	1.00	8.06	C
	ATOM	5726	CG	GLN	B	157	-13.830	14.779	20.448	1.00	8.73	C
	ATOM	5729	CD	GLN	B	157	-14.089	14.415	19.009	1.00	9.08	C
	ATOM	5730	OE1	GLN	B	157	-13.254	14.641	18.152	1.00	11.00	O
	ATOM	5731	NE2	GLN	B	157	-15.236	13.811	18.749	1.00	10.76	N
60	ATOM	5734	C	GLN	B	157	-11.462	17.344	22.077	1.00	7.12	C
	ATOM	5735	O	GLN	B	157	-11.942	18.287	22.701	1.00	7.80	O
	ATOM	5736	N	TYR	B	158	-10.267	17.376	21.508	1.00	7.08	N
	ATOM	5738	CA	TYR	B	158	-9.332	18.471	21.731	1.00	7.13	C
	ATOM	5740	CB	TYR	B	158	-8.677	18.365	23.128	1.00	6.96	C

	ATOM	5743	CG	TYR	B	158	-8.393	16.941	23.559	1.00	7.06	C
	ATOM	5744	CD1	TYR	B	158	-7.326	16.222	23.034	1.00	7.19	C
	ATOM	5746	CE1	TYR	B	158	-7.108	14.901	23.404	1.00	7.09	C
5	ATOM	5748	CZ	TYR	B	158	-7.946	14.281	24.320	1.00	7.18	C
	ATOM	5749	OH	TYR	B	158	-7.750	12.979	24.733	1.00	7.90	O
	ATOM	5751	CE2	TYR	B	158	-9.012	14.992	24.839	1.00	7.53	C
	ATOM	5753	CD2	TYR	B	158	-9.232	16.292	24.455	1.00	7.22	C
	ATOM	5755	C	TYR	B	158	-8.280	18.442	20.641	1.00	6.81	C
	ATOM	5756	O	TYR	B	158	-8.009	17.396	20.043	1.00	7.32	O
10	ATOM	5757	N	ALA	B	159	-7.670	19.601	20.393	1.00	7.32	N
	ATOM	5759	CA	ALA	B	159	-6.629	19.703	19.374	1.00	7.78	C
	ATOM	5761	CB	ALA	B	159	-6.680	21.052	18.663	1.00	8.55	C
	ATOM	5765	C	ALA	B	159	-5.229	19.451	19.903	1.00	7.90	C
	ATOM	5766	O	ALA	B	159	-4.297	19.384	19.110	1.00	8.83	O
15	ATOM	5767	N	MET	B	160	-5.055	19.302	21.220	1.00	7.36	N
	ATOM	5769	CA	MET	B	160	-3.713	19.139	21.772	1.00	7.42	C
	ATOM	5771	CB	MET	B	160	-3.733	18.986	23.293	1.00	8.13	C
	ATOM	5774	CG	MET	B	160	-4.060	20.269	24.029	1.00	8.32	C
	ATOM	5777	SD	MET	B	160	-5.812	20.754	24.003	1.00	8.06	S
20	ATOM	5778	CE	MET	B	160	-6.418	19.818	25.409	1.00	8.63	C
	ATOM	5782	C	MET	B	160	-3.042	17.927	21.119	1.00	7.49	C
	ATOM	5783	O	MET	B	160	-3.660	16.882	20.868	1.00	7.73	O
	ATOM	5784	N	ASP	B	161	-1.756	18.098	20.866	1.00	7.41	N
	ATOM	5786	CA	ASP	B	161	-0.986	17.115	20.130	1.00	7.72	C
25	ATOM	5788	CB	ASP	B	161	0.316	17.758	19.654	1.00	8.40	C
	ATOM	5791	CG	ASP	B	161	0.065	18.961	18.781	1.00	8.51	C
	ATOM	5792	OD1	ASP	B	161	0.577	20.072	19.078	1.00	9.92	O
	ATOM	5793	OD2	ASP	B	161	-0.668	18.829	17.794	1.00	8.73	O
	ATOM	5794	C	ASP	B	161	-0.704	15.870	20.953	1.00	7.16	C
30	ATOM	5795	O	ASP	B	161	-0.294	15.963	22.117	1.00	7.81	O
	ATOM	5796	N	THR	B	162	-0.901	14.722	20.319	1.00	7.20	N
	ATOM	5798	CA	THR	B	162	-0.669	13.420	20.924	1.00	7.17	C
	ATOM	5800	CB	THR	B	162	-1.969	12.811	21.499	1.00	7.29	C
	ATOM	5802	OG1	THR	B	162	-2.905	12.578	20.436	1.00	7.90	O
35	ATOM	5804	CG2	THR	B	162	-2.645	13.727	22.509	1.00	8.12	C
	ATOM	5808	C	THR	B	162	-0.154	12.465	19.857	1.00	7.27	C
	ATOM	5809	O	THR	B	162	-0.332	12.693	18.664	1.00	7.57	O
	ATOM	5810	N	TYR	B	163	0.414	11.350	20.298	1.00	7.74	N
	ATOM	5812	CA	TYR	B	163	0.840	10.282	19.401	1.00	7.85	C
40	ATOM	5814	CB	TYR	B	163	2.316	10.465	19.013	1.00	8.00	C
	ATOM	5817	CG	TYR	B	163	2.766	9.721	17.771	1.00	8.39	C
	ATOM	5818	CD1	TYR	B	163	2.621	10.309	16.533	1.00	10.58	C
	ATOM	5820	CE1	TYR	B	163	3.039	9.684	15.385	1.00	11.78	C
	ATOM	5822	CZ	TYR	B	163	3.642	8.452	15.458	1.00	11.29	C
45	ATOM	5823	OH	TYR	B	163	4.037	7.861	14.280	1.00	14.10	O
	ATOM	5825	CE2	TYR	B	163	3.807	7.835	16.689	1.00	9.46	C
	ATOM	5827	CD2	TYR	B	163	3.390	8.484	17.833	1.00	8.33	C
	ATOM	5829	C	TYR	B	163	0.643	8.948	20.088	1.00	7.90	C
	ATOM	5830	O	TYR	B	163	0.537	8.870	21.310	1.00	8.15	O
50	ATOM	5831	N	GLY	B	164	0.628	7.881	19.296	1.00	7.76	N
	ATOM	5833	CA	GLY	B	164	0.677	6.530	19.828	1.00	8.11	C
	ATOM	5836	C	GLY	B	164	1.667	6.417	20.964	1.00	7.49	C
	ATOM	5837	O	GLY	B	164	2.773	6.926	20.880	1.00	8.72	O
	ATOM	5838	N	GLY	B	165	1.262	5.708	22.009	1.00	7.45	N
55	ATOM	5840	CA	GLY	B	165	1.988	5.644	23.269	1.00	7.27	C
	ATOM	5843	C	GLY	B	165	1.350	6.507	24.339	1.00	6.66	C
	ATOM	5844	O	GLY	B	165	1.461	6.214	25.531	1.00	7.23	O
	ATOM	5845	N	GLN	B	166	0.662	7.572	23.923	1.00	6.83	N
	ATOM	5847	CA	GLN	B	166	-0.003	8.463	24.859	1.00	6.61	C
60	ATOM	5849	CB	GLN	B	166	0.045	9.919	24.381	1.00	6.74	C
	ATOM	5852	CG	GLN	B	166	1.451	10.489	24.459	1.00	7.33	C
	ATOM	5855	CD	GLN	B	166	1.507	11.943	24.074	1.00	6.80	C
	ATOM	5856	OE1	GLN	B	166	1.609	12.277	22.895	1.00	7.44	O
	ATOM	5857	NE2	GLN	B	166	1.421	12.831	25.056	1.00	8.44	N

	ATOM	5860	C	GLN	B	166	-1.429	8.054	25.211	1.00	6.58	C
	ATOM	5861	O	GLN	B	166	-2.003	8.683	26.096	1.00	6.83	O
	ATOM	5862	N	ALA	B	167	-2.023	7.043	24.587	1.00	6.87	N
5	ATOM	5864	CA	ALA	B	167	-3.285	6.550	25.133	1.00	6.77	C
	ATOM	5866	CB	ALA	B	167	-3.854	5.379	24.386	1.00	7.55	C
	ATOM	5870	C	ALA	B	167	-3.038	6.162	26.587	1.00	6.83	C
	ATOM	5871	O	ALA	B	167	-1.998	5.619	26.939	1.00	7.16	O
	ATOM	5872	N	GLY	B	168	-4.029	6.461	27.410	1.00	6.56	N
10	ATOM	5874	CA	GLY	B	168	-3.940	6.275	28.838	1.00	6.85	C
	ATOM	5877	C	GLY	B	168	-3.482	7.506	29.584	1.00	6.95	C
	ATOM	5878	O	GLY	B	168	-3.573	7.528	30.811	1.00	8.08	O
	ATOM	5879	N	SER	B	169	-2.983	8.524	28.883	1.00	6.66	N
	ATOM	5881	CA	SER	B	169	-2.522	9.730	29.550	1.00	6.73	C
	ATOM	5883	CB	SER	B	169	-1.937	10.743	28.561	1.00	6.97	C
15	ATOM	5886	OG	SER	B	169	-0.786	10.274	27.893	1.00	7.01	O
	ATOM	5888	C	SER	B	169	-3.682	10.418	30.252	1.00	6.37	C
	ATOM	5889	O	SER	B	169	-4.809	10.457	29.735	1.00	7.00	O
	ATOM	5890	N	PRO	B	170	-3.423	11.031	31.401	1.00	6.55	N
20	ATOM	5891	CA	PRO	B	170	-4.460	11.849	32.024	1.00	6.89	C
	ATOM	5893	CB	PRO	B	170	-3.857	12.207	33.376	1.00	7.40	C
	ATOM	5896	CG	PRO	B	170	-2.372	12.206	33.129	1.00	7.51	C
	ATOM	5899	CD	PRO	B	170	-2.132	11.112	32.117	1.00	7.19	C
	ATOM	5902	C	PRO	B	170	-4.681	13.102	31.183	1.00	6.85	C
25	ATOM	5903	O	PRO	B	170	-3.735	13.676	30.622	1.00	7.40	O
	ATOM	5904	N	VAL	B	171	-5.937	13.524	31.132	1.00	7.09	N
	ATOM	5906	CA	VAL	B	171	-6.348	14.785	30.543	1.00	7.34	C
	ATOM	5908	CB	VAL	B	171	-7.465	14.557	29.506	1.00	7.54	C
	ATOM	5910	CG1	VAL	B	171	-7.909	15.888	28.901	1.00	8.25	C
	ATOM	5914	CG2	VAL	B	171	-7.031	13.593	28.430	1.00	7.81	C
30	ATOM	5918	C	VAL	B	171	-6.840	15.593	31.737	1.00	7.34	C
	ATOM	5919	O	VAL	B	171	-7.955	15.357	32.214	1.00	8.22	O
	ATOM	5920	N	PHE	B	172	-5.982	16.449	32.278	1.00	7.59	N
	ATOM	5922	CA	PHE	B	172	-6.163	16.916	33.647	1.00	7.79	C
	ATOM	5924	CB	PHE	B	172	-5.221	16.170	34.623	1.00	8.27	C
35	ATOM	5927	CG	PHE	B	172	-3.744	16.499	34.490	1.00	8.37	C
	ATOM	5928	CD1	PHE	B	172	-3.131	17.378	35.375	1.00	9.16	C
	ATOM	5930	CE1	PHE	B	172	-1.781	17.635	35.304	1.00	9.65	C
	ATOM	5932	CZ	PHE	B	172	-1.013	17.033	34.328	1.00	9.66	C
	ATOM	5934	CE2	PHE	B	172	-1.601	16.164	33.436	1.00	9.16	C
40	ATOM	5936	CD2	PHE	B	172	-2.958	15.881	33.524	1.00	8.25	C
	ATOM	5938	C	PHE	B	172	-6.001	18.406	33.814	1.00	8.22	C
	ATOM	5939	O	PHE	B	172	-5.216	19.061	33.133	1.00	8.32	O
	ATOM	5940	N	GLU	B	173	-6.748	18.939	34.765	1.00	9.45	N
45	ATOM	5942	CA	GLU	B	173	-6.530	20.289	35.261	1.00	10.25	C
	ATOM	5944	CB	GLU	B	173	-7.785	20.812	35.938	1.00	10.74	C
	ATOM	5947	CG	GLU	B	173	-8.990	20.794	35.029	1.00	11.64	C
	ATOM	5950	CD	GLU	B	173	-10.231	21.270	35.737	1.00	12.37	C
	ATOM	5951	OE1	GLU	B	173	-10.771	22.325	35.349	1.00	13.46	O
	ATOM	5952	OE2	GLU	B	173	-10.643	20.583	36.698	1.00	14.40	O
50	ATOM	5953	C	GLU	B	173	-5.379	20.263	36.258	1.00	11.18	C
	ATOM	5954	O	GLU	B	173	-5.337	19.402	37.127	1.00	11.76	O
	ATOM	5955	N	GLN	B	174	-4.454	21.209	36.145	1.00	12.69	N
	ATOM	5957	CA	GLN	B	174	-3.289	21.244	37.026	1.00	14.03	C
	ATOM	5959	CB	GLN	B	174	-2.344	22.376	36.616	1.00	14.32	C
55	ATOM	5962	CG	GLN	B	174	-1.682	22.176	35.261	1.00	14.57	C
	ATOM	5965	CD	GLN	B	174	-0.500	21.229	35.272	1.00	13.85	C
	ATOM	5966	OE1	GLN	B	174	-0.120	20.709	34.207	1.00	14.35	O
	ATOM	5967	NE2	GLN	B	174	0.089	20.999	36.440	1.00	13.83	N
	ATOM	5970	C	GLN	B	174	-3.670	21.420	38.499	1.00	14.98	C
60	ATOM	5971	O	GLN	B	174	-3.055	20.828	39.382	1.00	15.06	O
	ATOM	5972	N	SER	B	175	-4.688	22.232	38.754	1.00	16.64	N
	ATOM	5974	CA	SER	B	175	-5.086	22.556	40.114	1.00	18.96	C
	ATOM	5976	CB	SER	B	175	-4.237	23.718	40.627	1.00	19.69	C
	ATOM	5979	OG	SER	B	175	-4.601	24.095	41.945	1.00	22.47	O

	ATOM	5981	C	SER B 175	-6.561	22.930	40.126	1.00	19.54	C
	ATOM	5982	O	SER B 175	-6.933	24.006	39.666	1.00	20.78	O
	ATOM	5983	N	SER B 176	-7.400	22.039	40.644	1.00	19.58	N
5	ATOM	5985	CA	SER B 176	-8.842	22.251	40.640	1.00	20.22	C
	ATOM	5987	CB	SER B 176	-9.468	21.458	39.495	1.00	20.96	C
	ATOM	5990	OG	SER B 176	-10.867	21.629	39.459	1.00	23.01	O
	ATOM	5992	C	SER B 176	-9.475	21.805	41.947	1.00	20.01	C
	ATOM	5993	O	SER B 176	-8.995	20.878	42.599	1.00	18.97	O
	ATOM	5994	N	SER B 177	-10.560	22.486	42.311	1.00	20.67	N
10	ATOM	5996	CA	SER B 177	-11.457	22.038	43.368	1.00	21.82	C
	ATOM	5998	CB	SER B 177	-11.738	23.164	44.369	1.00	22.03	C
	ATOM	6001	OG	SER B 177	-12.180	24.343	43.719	1.00	24.65	O
	ATOM	6003	C	SER B 177	-12.749	21.547	42.706	1.00	22.02	C
	ATOM	6004	O	SER B 177	-13.563	22.340	42.230	1.00	23.51	O
15	ATOM	6005	N	ARG B 178	-12.881	20.226	42.622	1.00	21.68	N
	ATOM	6007	CA	ARG B 178	-14.097	19.547	42.176	1.00	20.93	C
	ATOM	6009	CB	ARG B 178	-13.937	18.996	40.745	1.00	20.43	C
	ATOM	6012	CG	ARG B 178	-13.783	20.018	39.627	1.00	18.45	C
	ATOM	6015	CD	ARG B 178	-13.677	19.382	38.238	1.00	16.30	C
20	ATOM	6018	NE	ARG B 178	-13.336	20.340	37.188	1.00	15.06	N
	ATOM	6020	CZ	ARG B 178	-14.210	20.982	36.429	1.00	15.42	C
	ATOM	6021	NH1	ARG B 178	-15.520	20.830	36.599	1.00	16.79	N
	ATOM	6024	NH2	ARG B 178	-13.766	21.800	35.487	1.00	15.73	N
	ATOM	6027	C	ARG B 178	-14.317	18.378	43.127	1.00	21.07	C
25	ATOM	6028	O	ARG B 178	-13.498	18.130	44.007	1.00	21.95	O
	ATOM	6029	N	THR B 179	-15.409	17.643	42.952	1.00	20.41	N
	ATOM	6031	CA	THR B 179	-15.601	16.424	43.723	1.00	20.45	C
	ATOM	6033	CB	THR B 179	-16.934	15.754	43.349	1.00	21.16	C
	ATOM	6035	OG1	THR B 179	-18.030	16.605	43.717	1.00	22.79	O
30	ATOM	6037	CG2	THR B 179	-17.156	14.483	44.160	1.00	22.10	C
	ATOM	6041	C	THR B 179	-14.439	15.480	43.434	1.00	19.46	C
	ATOM	6042	O	THR B 179	-14.150	15.185	42.267	1.00	19.41	O
	ATOM	6043	N	ASN B 180	-13.759	15.050	44.493	1.00	18.57	N
	ATOM	6045	CA	ASN B 180	-12.593	14.162	44.410	1.00	18.41	C
35	ATOM	6047	CB	ASN B 180	-12.948	12.851	43.684	1.00	18.60	C
	ATOM	6050	CG	ASN B 180	-11.881	11.765	43.846	1.00	18.64	C
	ATOM	6051	OD1	ASN B 180	-11.492	11.110	42.874	1.00	17.95	O
	ATOM	6052	ND2	ASN B 180	-11.407	11.572	45.071	1.00	19.48	N
	ATOM	6055	C	ASN B 180	-11.376	14.840	43.778	1.00	17.73	C
40	ATOM	6056	O	ASN B 180	-10.477	14.160	43.272	1.00	18.18	O
	ATOM	6057	N	CYS B 181	-11.329	16.175	43.845	1.00	18.01	N
	ATOM	6059	CA	CYS B 181	-10.170	16.955	43.412	1.00	17.47	C
	ATOM	6061	CB	CYS B 181	-10.365	17.519	42.007	1.00	16.55	C
	ATOM	6064	SG	CYS B 181	-10.449	16.203	40.788	1.00	14.03	S
45	ATOM	6065	C	CYS B 181	-9.864	18.092	44.372	1.00	18.41	C
	ATOM	6066	O	CYS B 181	-10.756	18.845	44.780	1.00	19.23	O
	ATOM	6067	N	ASN B 182	-8.595	18.188	44.734	1.00	19.10	N
	ATOM	6069	CA	ASN B 182	-8.057	19.316	45.475	1.00	19.72	C
	ATOM	6071	CB	ASN B 182	-8.215	19.085	46.989	1.00	20.52	C
50	ATOM	6074	CG	ASN B 182	-7.873	20.313	47.824	0.50	21.48	C
	ATOM	6075	OD1	ASN B 182	-7.469	20.192	48.983	0.50	23.03	O
	ATOM	6076	ND2	ASN B 182	-8.051	21.498	47.248	0.50	22.48	N
	ATOM	6079	C	ASN B 182	-6.593	19.428	45.053	1.00	19.26	C
	ATOM	6080	O	ASN B 182	-5.683	19.248	45.854	1.00	20.75	O
55	ATOM	6081	N	GLY B 183	-6.392	19.722	43.767	1.00	17.83	N
	ATOM	6083	CA	GLY B 183	-5.102	19.586	43.106	1.00	16.31	C
	ATOM	6086	C	GLY B 183	-5.308	19.065	41.691	1.00	15.03	C
	ATOM	6087	O	GLY B 183	-6.328	19.348	41.063	1.00	15.07	O
	ATOM	6088	N	PRO B 184	-4.353	18.300	41.168	1.00	13.71	N
60	ATOM	6089	CA	PRO B 184	-4.487	17.759	39.810	1.00	12.66	C
	ATOM	6091	CB	PRO B 184	-3.241	16.889	39.651	1.00	13.12	C
	ATOM	6094	CG	PRO B 184	-2.259	17.444	40.640	1.00	14.51	C
	ATOM	6097	CD	PRO B 184	-3.077	17.925	41.800	1.00	14.52	C
	ATOM	6100	C	PRO B 184	-5.769	16.941	39.671	1.00	11.46	C

5	ATOM	6101	O	PRO	B	184	-6.076	16.141	40.565	1.00	12.12	O
	ATOM	6102	N	CYS	B	185	-6.500	17.154	38.581	1.00	10.93	N
	ATOM	6104	CA	CYS	B	185	-7.851	16.641	38.453	1.00	10.37	C
	ATOM	6106	CB	CYS	B	185	-8.838	17.758	38.780	1.00	11.29	C
	ATOM	6109	SG	CYS	B	185	-10.536	17.205	38.967	1.00	13.15	S
	ATOM	6110	C	CYS	B	185	-8.095	16.139	37.046	1.00	9.18	C
	ATOM	6111	O	CYS	B	185	-8.272	16.933	36.118	1.00	9.91	O
	ATOM	6112	N	SER	B	186	-8.075	14.824	36.874	1.00	9.10	N
10	ATOM	6114	CA	SER	B	186	-8.312	14.244	35.555	1.00	8.73	C
	ATOM	6116	CB	SER	B	186	-7.828	12.808	35.521	1.00	9.22	C
	ATOM	6119	OG	SER	B	186	-6.445	12.784	35.662	1.00	10.95	O
	ATOM	6121	C	SER	B	186	-9.792	14.276	35.205	1.00	8.85	C
15	ATOM	6122	O	SER	B	186	-10.631	13.896	36.021	1.00	9.47	O
	ATOM	6123	N	LEU	B	187	-10.070	14.716	33.981	1.00	8.48	N
	ATOM	6125	CA	LEU	B	187	-11.417	14.803	33.438	1.00	8.48	C
	ATOM	6127	CB	LEU	B	187	-11.672	16.220	32.909	1.00	8.73	C
	ATOM	6130	CG	LEU	B	187	-11.486	17.345	33.923	1.00	9.71	C
20	ATOM	6132	CD1	LEU	B	187	-11.768	18.686	33.266	1.00	10.15	C
	ATOM	6136	CD2	LEU	B	187	-12.372	17.141	35.147	1.00	11.50	C
	ATOM	6140	C	LEU	B	187	-11.677	13.791	32.329	1.00	8.23	C
	ATOM	6141	O	LEU	B	187	-12.828	13.573	31.958	1.00	8.64	O
	ATOM	6142	N	ALA	B	188	-10.612	13.181	31.805	1.00	8.18	N
25	ATOM	6144	CA	ALA	B	188	-10.694	12.216	30.727	1.00	7.99	C
	ATOM	6146	CB	ALA	B	188	-10.950	12.910	29.397	1.00	7.96	C
	ATOM	6150	C	ALA	B	188	-9.385	11.425	30.699	1.00	7.51	C
	ATOM	6151	O	ALA	B	188	-8.414	11.769	31.366	1.00	7.88	O
	ATOM	6152	N	VAL	B	189	-9.389	10.372	29.896	1.00	7.53	N
30	ATOM	6154	CA	VAL	B	189	-8.217	9.552	29.624	1.00	7.68	C
	ATOM	6156	CB	BVAL	B	189	-8.268	8.135	30.229	0.35	8.28	C
	ATOM	6157	CB	AVAL	B	189	-8.511	8.063	29.995	0.65	8.30	C
	ATOM	6160	CG1BVAL	B	189	-9.551	7.453	29.930	0.35	9.36	C	
	ATOM	6161	CG1AVAL	B	189	-7.306	7.209	29.742	0.65	9.65	C	
35	ATOM	6168	CG2BVAL	B	189	-7.113	7.296	29.717	0.35	10.02	C	
	ATOM	6169	CG2AVAL	B	189	-8.970	7.917	31.433	0.65	8.62	C	
	ATOM	6176	C	VAL	B	189	-7.982	9.584	28.117	1.00	7.27	C
	ATOM	6177	O	VAL	B	189	-8.890	9.267	27.338	1.00	7.48	O
	ATOM	6178	N	HIS	B	190	-6.793	9.991	27.673	1.00	6.91	N
40	ATOM	6180	CA	HIS	B	190	-6.546	10.097	26.248	1.00	6.84	C
	ATOM	6182	CB	HIS	B	190	-5.167	10.736	25.956	1.00	6.94	C
	ATOM	6185	CG	HIS	B	190	-4.917	10.787	24.504	1.00	6.68	C
	ATOM	6186	ND1	HIS	B	190	-5.791	11.423	23.659	1.00	7.50	N
	ATOM	6188	CE1	HIS	B	190	-5.449	11.150	22.417	1.00	7.27	C
45	ATOM	6190	NE2	HIS	B	190	-4.369	10.394	22.428	1.00	7.70	N
	ATOM	6192	CD2	HIS	B	190	-4.006	10.160	23.732	1.00	7.53	C
	ATOM	6194	C	HIS	B	190	-6.656	8.714	25.580	1.00	6.62	C
	ATOM	6195	O	HIS	B	190	-6.168	7.735	26.122	1.00	7.01	O
	ATOM	6196	N	THR	B	191	-7.271	8.655	24.402	1.00	6.90	N
50	ATOM	6198	CA	THR	B	191	-7.429	7.367	23.723	1.00	7.18	C
	ATOM	6200	CB	THR	B	191	-8.815	6.739	23.986	1.00	7.59	C
	ATOM	6202	OG1	THR	B	191	-9.845	7.700	23.751	1.00	9.16	O
	ATOM	6204	CG2	THR	B	191	-8.974	6.296	25.430	1.00	8.33	C
	ATOM	6208	C	THR	B	191	-7.162	7.340	22.221	1.00	7.39	C
55	ATOM	6209	O	THR	B	191	-6.635	6.336	21.746	1.00	8.24	O
	ATOM	6210	N	ASN	B	192	-7.589	8.362	21.472	1.00	7.54	N
	ATOM	6212	CA	ASN	B	192	-7.637	8.270	20.016	1.00	8.61	C
	ATOM	6214	CB	ASN	B	192	-9.084	8.158	19.500	1.00	10.14	C
	ATOM	6217	CG	ASN	B	192	-9.884	7.097	20.205	1.00	13.02	C
60	ATOM	6218	OD1	ASN	B	192	-9.925	5.949	19.768	1.00	17.47	O
	ATOM	6219	ND2	ASN	B	192	-10.571	7.484	21.269	1.00	13.77	N
	ATOM	6222	C	ASN	B	192	-7.053	9.497	19.349	1.00	7.81	C
	ATOM	6223	O	ASN	B	192	-7.187	10.604	19.845	1.00	7.54	O
	ATOM	6224	N	GLY	B	193	-6.466	9.272	18.178	1.00	7.89	N
	ATOM	6226	CA	GLY	B	193	-6.097	10.347	17.282	1.00	7.94	C
	ATOM	6229	C	GLY	B	193	-7.269	10.780	16.424	1.00	7.68	C

	ATOM	6230	O	GLY	B	193	-8.434	10.495	16.712	1.00	8.59	O
	ATOM	6231	N	VAL	B	194	-6.934	11.448	15.329	1.00	7.94	N
	ATOM	6233	CA	VAL	B	194	-7.905	12.057	14.430	1.00	8.60	C
	ATOM	6235	CB	VAL	B	194	-7.210	13.166	13.608	1.00	9.21	C
5	ATOM	6237	CG1	VAL	B	194	-8.096	13.671	12.465	1.00	10.61	C
	ATOM	6241	CG2	VAL	B	194	-6.800	14.308	14.504	1.00	9.07	C
	ATOM	6245	C	VAL	B	194	-8.484	10.982	13.518	1.00	9.30	C
	ATOM	6246	O	VAL	B	194	-7.749	10.269	12.840	1.00	10.19	O
	ATOM	6247	N	TYR	B	195	-9.806	10.861	13.489	1.00	9.47	N
10	ATOM	6249	CA	TYR	B	195	-10.480	9.922	12.601	1.00	10.28	C
	ATOM	6251	CB	TYR	B	195	-10.327	8.471	13.092	1.00	11.06	C
	ATOM	6254	CG	TYR	B	195	-11.205	8.082	14.268	1.00	11.54	C
	ATOM	6255	CD1	TYR	B	195	-10.850	8.436	15.562	1.00	11.78	C
	ATOM	6257	CE1	TYR	B	195	-11.625	8.075	16.647	1.00	13.23	C
15	ATOM	6259	CZ	TYR	B	195	-12.799	7.391	16.439	1.00	14.48	C
	ATOM	6260	OH	TYR	B	195	-13.573	7.033	17.518	1.00	17.11	O
	ATOM	6262	CE2	TYR	B	195	-13.188	7.044	15.160	1.00	15.20	C
	ATOM	6264	CD2	TYR	B	195	-12.393	7.385	14.082	1.00	13.19	C
	ATOM	6266	C	TYR	B	195	-11.953	10.279	12.489	1.00	10.34	C
20	ATOM	6267	O	TYR	B	195	-12.463	11.132	13.209	1.00	10.36	O
	ATOM	6268	N	GLY	B	196	-12.644	9.603	11.582	1.00	11.32	N
	ATOM	6270	CA	GLY	B	196	-14.087	9.629	11.600	1.00	11.81	C
	ATOM	6273	C	GLY	B	196	-14.742	10.932	11.216	1.00	11.29	C
	ATOM	6274	O	GLY	B	196	-15.881	11.184	11.604	1.00	12.54	O
25	ATOM	6275	N	GLY	B	197	-14.038	11.749	10.452	1.00	11.14	N
	ATOM	6277	CA	GLY	B	197	-14.556	13.043	10.072	1.00	11.40	C
	ATOM	6280	C	GLY	B	197	-14.354	14.124	11.118	1.00	10.86	C
	ATOM	6281	O	GLY	B	197	-14.712	15.268	10.864	1.00	11.89	O
	ATOM	6282	N	SER	B	198	-13.756	13.794	12.260	1.00	10.19	N
30	ATOM	6284	CA	SER	B	198	-13.394	14.795	13.240	1.00	9.83	C
	ATOM	6286	CB	SER	B	198	-13.303	14.175	14.624	1.00	9.77	C
	ATOM	6289	OG	SER	B	198	-12.942	15.156	15.567	1.00	9.88	O
	ATOM	6291	C	SER	B	198	-12.066	15.428	12.891	1.00	10.01	C
	ATOM	6292	O	SER	B	198	-11.212	14.812	12.266	1.00	12.17	O
35	ATOM	6293	N	SER	B	199	-11.898	16.664	13.339	1.00	9.97	N
	ATOM	6295	CA	SER	B	199	-10.645	17.378	13.227	1.00	10.64	C
	ATOM	6297	CB	SER	B	199	-10.911	18.863	12.962	1.00	11.88	C
	ATOM	6300	OG	SER	B	199	-11.618	19.054	11.760	1.00	15.50	O
	ATOM	6302	C	SER	B	199	-9.791	17.257	14.486	1.00	9.48	C
40	ATOM	6303	O	SER	B	199	-8.720	17.848	14.532	1.00	10.66	O
	ATOM	6304	N	TYR	B	200	-10.239	16.495	15.480	1.00	8.05	N
	ATOM	6306	CA	TYR	B	200	-9.643	16.523	16.805	1.00	7.75	C
	ATOM	6308	CB	TYR	B	200	-10.654	17.084	17.810	1.00	7.69	C
	ATOM	6311	CG	TYR	B	200	-11.101	18.490	17.511	1.00	8.31	C
45	ATOM	6312	CD1	TYR	B	200	-10.287	19.570	17.798	1.00	9.21	C
	ATOM	6314	CE1	TYR	B	200	-10.680	20.867	17.534	1.00	10.29	C
	ATOM	6316	CZ	TYR	B	200	-11.910	21.112	16.988	1.00	10.84	C
	ATOM	6317	OH	TYR	B	200	-12.299	22.414	16.730	1.00	13.51	O
	ATOM	6319	CE2	TYR	B	200	-12.751	20.065	16.697	1.00	11.29	C
50	ATOM	6321	CD2	TYR	B	200	-12.345	18.748	16.960	1.00	10.10	C
	ATOM	6323	C	TYR	B	200	-9.217	15.133	17.266	1.00	7.26	C
	ATOM	6324	O	TYR	B	200	-9.662	14.114	16.746	1.00	8.16	O
	ATOM	6325	N	ASN	B	201	-8.348	15.125	18.274	1.00	7.06	N
	ATOM	6327	CA	ASN	B	201	-8.042	13.952	19.084	1.00	7.16	C
55	ATOM	6329	CB	ASN	B	201	-6.680	14.153	19.748	1.00	7.15	C
	ATOM	6332	CG	ASN	B	201	-5.554	14.230	18.742	1.00	7.21	C
	ATOM	6333	OD1	ASN	B	201	-5.516	13.447	17.803	1.00	7.69	O
	ATOM	6334	ND2	ASN	B	201	-4.644	15.175	18.926	1.00	7.72	N
	ATOM	6337	C	ASN	B	201	-9.132	13.735	20.118	1.00	7.29	C
60	ATOM	6338	O	ASN	B	201	-9.912	14.647	20.394	1.00	7.53	O
	ATOM	6339	N	ARG	B	202	-9.206	12.536	20.697	1.00	6.98	N
	ATOM	6341	CA	ARG	B	202	-10.279	12.211	21.629	1.00	7.41	C
	ATOM	6343	CB	ARG	B	202	-11.383	11.355	20.995	1.00	8.88	C
	ATOM	6346	CG	ARG	B	202	-11.693	11.653	19.568	1.00	9.57	C

	ATOM	6349	CD	ARG	B	202	-12.972	11.011	19.099	1.00	10.98	C
	ATOM	6352	NE	ARG	B	202	-13.038	11.045	17.669	1.00	10.86	N
	ATOM	6354	CZ	ARG	B	202	-14.060	10.645	16.946	1.00	10.42	C
5	ATOM	6355	NH1	ARG	B	202	-15.207	10.244	17.495	1.00	11.93	N
	ATOM	6358	NH2	ARG	B	202	-13.935	10.652	15.633	1.00	11.59	N
	ATOM	6361	C	ARG	B	202	-9.772	11.449	22.843	1.00	7.12	C
	ATOM	6362	O	ARG	B	202	-8.800	10.686	22.775	1.00	7.24	O
	ATOM	6363	N	GLY	B	203	-10.506	11.616	23.931	1.00	7.36	N
	ATOM	6365	CA	GLY	B	203	-10.273	10.888	25.156	1.00	7.64	C
10	ATOM	6368	C	GLY	B	203	-11.594	10.478	25.782	1.00	7.46	C
	ATOM	6369	O	GLY	B	203	-12.600	11.167	25.693	1.00	8.95	O
	ATOM	6370	N	THR	B	204	-11.601	9.321	26.422	1.00	7.91	N
	ATOM	6372	CA	THR	B	204	-12.766	8.862	27.169	1.00	7.85	C
	ATOM	6374	CB	THR	B	204	-12.526	7.440	27.646	1.00	7.99	C
15	ATOM	6376	OG1	THR	B	204	-12.283	6.626	26.490	1.00	9.14	O
	ATOM	6378	CG2	THR	B	204	-13.742	6.879	28.396	1.00	8.93	C
	ATOM	6382	C	THR	B	204	-13.049	9.778	28.339	1.00	7.48	C
	ATOM	6383	O	THR	B	204	-12.207	9.977	29.209	1.00	8.11	O
	ATOM	6384	N	ARG	B	205	-14.246	10.340	28.347	1.00	7.64	N
20	ATOM	6386	CA	ARG	B	205	-14.673	11.241	29.393	1.00	8.00	C
	ATOM	6388	CB	ARG	B	205	-15.976	11.911	28.965	1.00	8.79	C
	ATOM	6391	CG	ARG	B	205	-16.504	12.958	29.902	1.00	8.59	C
	ATOM	6394	CD	ARG	B	205	-17.749	13.634	29.351	1.00	9.13	C
	ATOM	6397	NE	ARG	B	205	-18.197	14.685	30.247	1.00	9.66	N
25	ATOM	6399	CZ	ARG	B	205	-19.108	15.593	29.932	1.00	11.19	C
	ATOM	6400	NH1	ARG	B	205	-19.463	16.494	30.836	1.00	12.79	N
	ATOM	6403	NH2	ARG	B	205	-19.631	15.622	28.720	1.00	12.68	N
	ATOM	6406	C	ARG	B	205	-14.893	10.499	30.697	1.00	8.17	C
	ATOM	6407	O	ARG	B	205	-15.442	9.398	30.704	1.00	8.50	O
30	ATOM	6408	N	ILE	B	206	-14.511	11.107	31.803	1.00	7.90	N
	ATOM	6410	CA	ILE	B	206	-14.857	10.543	33.102	1.00	8.22	C
	ATOM	6412	CB	ILE	B	206	-13.888	10.984	34.205	1.00	8.53	C
	ATOM	6414	CG1	ILE	B	206	-12.479	10.503	33.832	1.00	10.37	C
	ATOM	6417	CD1	ILE	B	206	-11.395	10.782	34.838	1.00	11.44	C
35	ATOM	6421	CG2	ILE	B	206	-14.335	10.417	35.576	1.00	8.96	C
	ATOM	6425	C	ILE	B	206	-16.304	10.954	33.378	1.00	8.18	C
	ATOM	6426	O	ILE	B	206	-16.577	12.055	33.837	1.00	9.63	O
	ATOM	6427	N	THR	B	207	-17.221	10.054	33.053	1.00	8.48	N
	ATOM	6429	CA	THR	B	207	-18.633	10.182	33.409	1.00	8.64	C
40	ATOM	6431	CB	THR	B	207	-19.500	9.287	32.520	1.00	9.02	C
	ATOM	6433	OG1	THR	B	207	-19.159	7.926	32.815	1.00	9.39	O
	ATOM	6435	CG2	THR	B	207	-19.290	9.543	31.017	1.00	9.86	C
	ATOM	6439	C	THR	B	207	-18.829	9.725	34.857	1.00	8.82	C
	ATOM	6440	O	THR	B	207	-17.906	9.220	35.505	1.00	9.18	O
45	ATOM	6441	N	LYS	B	208	-20.060	9.852	35.352	1.00	9.26	N
	ATOM	6443	CA	LYS	B	208	-20.369	9.298	36.665	1.00	9.84	C
	ATOM	6445	CB	LYS	B	208	-21.833	9.519	37.046	1.00	10.99	C
	ATOM	6448	CG	LYS	B	208	-22.087	9.129	38.528	1.00	14.71	C
	ATOM	6451	CD	LYS	B	208	-23.399	9.593	39.068	1.00	16.96	C
50	ATOM	6454	CE	LYS	B	208	-23.489	9.258	40.552	1.00	19.57	C
	ATOM	6457	NZ	LYS	B	208	-23.241	7.822	40.859	1.00	20.47	N
	ATOM	6461	C	LYS	B	208	-20.034	7.814	36.745	1.00	9.27	C
	ATOM	6462	O	LYS	B	208	-19.537	7.336	37.761	1.00	10.16	O
	ATOM	6463	N	GLU	B	209	-20.331	7.079	35.694	1.00	9.18	N
55	ATOM	6465	CA	GLU	B	209	-20.113	5.643	35.715	1.00	9.01	C
	ATOM	6467	CB	GLU	B	209	-20.903	4.935	34.624	1.00	9.53	C
	ATOM	6470	CG	GLU	B	209	-22.414	5.046	34.816	1.00	10.25	C
	ATOM	6473	CD	GLU	B	209	-22.978	6.405	34.428	1.00	10.76	C
	ATOM	6474	OE1	GLU	B	209	-23.862	6.914	35.155	1.00	12.33	O
60	ATOM	6475	OE2	GLU	B	209	-22.549	6.961	33.386	1.00	11.21	O
	ATOM	6476	C	GLU	B	209	-18.624	5.295	35.653	1.00	8.78	C
	ATOM	6477	O	GLU	B	209	-18.183	4.353	36.318	1.00	9.54	O
	ATOM	6478	N	VAL	B	210	-17.843	6.052	34.878	1.00	8.31	N
	ATOM	6480	CA	VAL	B	210	-16.392	5.869	34.868	1.00	8.33	C

	ATOM	6482	CB	VAL	B	210	-15.715	6.782	33.835	1.00	8.11	C
	ATOM	6484	CG1	VAL	B	210	-14.194	6.643	33.918	1.00	8.28	C
	ATOM	6488	CG2	VAL	B	210	-16.207	6.468	32.427	1.00	8.18	C
5	ATOM	6492	C	VAL	B	210	-15.835	6.162	36.264	1.00	8.18	C
	ATOM	6493	O	VAL	B	210	-15.034	5.400	36.807	1.00	8.46	O
	ATOM	6494	N	PHE	B	211	-16.257	7.285	36.828	1.00	8.80	N
	ATOM	6496	CA	PHE	B	211	-15.865	7.717	38.169	1.00	8.95	C
	ATOM	6498	CB	PHE	B	211	-16.632	8.996	38.522	1.00	9.38	C
10	ATOM	6501	CG	PHE	B	211	-16.350	9.534	39.891	1.00	10.33	C
	ATOM	6502	CD1	PHE	B	211	-17.036	9.054	40.992	1.00	12.42	C
	ATOM	6504	CE1	PHE	B	211	-16.794	9.562	42.250	1.00	14.20	C
	ATOM	6506	CZ	PHE	B	211	-15.867	10.570	42.422	1.00	14.22	C
	ATOM	6508	CE2	PHE	B	211	-15.184	11.071	41.328	1.00	12.56	C
	ATOM	6510	CD2	PHE	B	211	-15.427	10.548	40.077	1.00	10.94	C
15	ATOM	6512	C	PHE	B	211	-16.144	6.610	39.183	1.00	9.01	C
	ATOM	6513	O	PHE	B	211	-15.284	6.254	39.997	1.00	9.62	O
	ATOM	6514	N	ASP	B	212	-17.341	6.057	39.145	1.00	9.24	N
	ATOM	6516	CA	ASP	B	212	-17.719	5.020	40.091	1.00	9.38	C
20	ATOM	6518	CB	ASP	B	212	-19.220	4.742	40.000	1.00	10.22	C
	ATOM	6521	CG	ASP	B	212	-20.081	5.866	40.585	1.00	10.96	C
	ATOM	6522	OD1	ASP	B	212	-19.596	6.712	41.352	1.00	12.82	O
	ATOM	6523	OD2	ASP	B	212	-21.294	5.924	40.326	1.00	14.21	O
	ATOM	6524	C	ASP	B	212	-16.920	3.730	39.883	1.00	9.20	C
25	ATOM	6525	O	ASP	B	212	-16.558	3.075	40.860	1.00	9.67	O
	ATOM	6526	N	ASN	B	213	-16.642	3.364	38.646	1.00	8.79	N
	ATOM	6528	CA	ASN	B	213	-15.823	2.182	38.386	1.00	8.75	C
	ATOM	6530	CB	BASN	B	213	-15.892	1.765	36.925	0.35	8.75	C
	ATOM	6531	CB	AASN	B	213	-15.742	1.816	36.880	0.65	8.93	C
30	ATOM	6536	CG	BASN	B	213	-17.240	1.173	36.556	0.35	9.22	C
	ATOM	6537	CG	AASN	B	213	-16.833	0.837	36.379	0.65	9.63	C
	ATOM	6538	OD1BASN	B	213	-17.635	1.198	35.396	0.35	11.25	O	
	ATOM	6539	OD1AASN	B	213	-17.182	0.862	35.191	0.65	11.89	O	
	ATOM	6540	ND2BASN	B	213	-17.948	0.634	37.537	0.35	8.61	N	
35	ATOM	6541	ND2AASN	B	213	-17.315	-0.040	37.230	0.65	9.89	N	
	ATOM	6546	C	ASN	B	213	-14.385	2.380	38.876	1.00	8.12	C
	ATOM	6547	O	ASN	B	213	-13.866	1.530	39.585	1.00	8.56	O
	ATOM	6548	N	LEU	B	214	-13.754	3.499	38.509	1.00	8.05	N
	ATOM	6550	CA	LEU	B	214	-12.388	3.756	38.975	1.00	8.09	C
40	ATOM	6552	CB	LEU	B	214	-11.878	5.101	38.472	1.00	8.15	C
	ATOM	6555	CG	LEU	B	214	-11.645	5.232	36.974	1.00	8.61	C
	ATOM	6557	CD1	LEU	B	214	-11.247	6.665	36.638	1.00	9.83	C
	ATOM	6561	CD2	LEU	B	214	-10.596	4.247	36.475	1.00	9.91	C
	ATOM	6565	C	LEU	B	214	-12.321	3.712	40.498	1.00	8.01	C
45	ATOM	6566	O	LEU	B	214	-11.378	3.153	41.070	1.00	8.50	O
	ATOM	6567	N	THR	B	215	-13.313	4.314	41.144	1.00	8.38	N
	ATOM	6569	CA	THR	B	215	-13.315	4.376	42.596	1.00	8.79	C
	ATOM	6571	CB	THR	B	215	-14.418	5.334	43.053	1.00	9.27	C
	ATOM	6573	OG1	THR	B	215	-14.177	6.633	42.485	1.00	10.10	O
50	ATOM	6575	CG2	THR	B	215	-14.416	5.517	44.571	1.00	11.07	C
	ATOM	6579	C	THR	B	215	-13.481	2.979	43.209	1.00	8.80	C
	ATOM	6580	O	THR	B	215	-12.791	2.625	44.166	1.00	9.21	O
	ATOM	6581	N	ASN	B	216	-14.370	2.179	42.646	1.00	8.57	N
	ATOM	6583	CA	ASN	B	216	-14.557	0.818	43.115	1.00	8.67	C
55	ATOM	6585	CB	ASN	B	216	-15.734	0.173	42.381	1.00	9.22	C
	ATOM	6588	CG	ASN	B	216	-15.982	-1.271	42.786	1.00	8.90	C
	ATOM	6589	OD1	ASN	B	216	-15.870	-1.642	43.963	1.00	9.87	O
	ATOM	6590	ND2	ASN	B	216	-16.303	-2.099	41.811	1.00	10.95	N
	ATOM	6593	C	ASN	B	216	-13.273	-0.002	42.920	1.00	8.34	C
60	ATOM	6594	O	ASN	B	216	-12.861	-0.759	43.806	1.00	8.95	O
	ATOM	6595	N	TRP	B	217	-12.626	0.156	41.771	1.00	8.60	N
	ATOM	6597	CA	TRP	B	217	-11.442	-0.632	41.484	1.00	8.62	C
	ATOM	6599	CB	TRP	B	217	-11.051	-0.484	40.025	1.00	8.99	C
	ATOM	6602	CG	TRP	B	217	-12.086	-0.995	39.080	1.00	9.18	C
	ATOM	6603	CD1	TRP	B	217	-13.046	-1.934	39.324	1.00	10.15	C

5	ATOM	6605	NE1	TRP	B	217	-13.804	-2.145	38.197	1.00	11.24	N
	ATOM	6607	CE2	TRP	B	217	-13.350	-1.320	37.207	1.00	10.18	C
	ATOM	6608	CD2	TRP	B	217	-12.272	-0.584	37.733	1.00	9.00	C
	ATOM	6609	CE3	TRP	B	217	-11.640	0.346	36.907	1.00	9.73	C
	ATOM	6611	CZ3	TRP	B	217	-12.074	0.488	35.602	1.00	11.01	C
	ATOM	6613	CH2	TRP	B	217	-13.139	-0.262	35.117	1.00	11.64	C
	ATOM	6615	CZ2	TRP	B	217	-13.799	-1.163	35.897	1.00	11.72	C
	ATOM	6617	C	TRP	B	217	-10.303	-0.253	42.431	1.00	8.36	C
10	ATOM	6618	O	TRP	B	217	-9.603	-1.117	42.953	1.00	8.91	O
	ATOM	6619	N	LYS	B	218	-10.123	1.033	42.695	1.00	8.79	N
	ATOM	6621	CA	LYS	B	218	-9.100	1.444	43.625	1.00	9.20	C
	ATOM	6623	CB	LYS	B	218	-8.827	2.934	43.515	1.00	11.18	C
15	ATOM	6626	CG	LYS	B	218	-9.737	3.843	44.197	1.00	15.17	C
	ATOM	6629	CD	LYS	B	218	-9.326	5.287	43.946	1.00	18.59	C
	ATOM	6632	CE	LYS	B	218	-10.240	6.273	44.642	1.00	20.56	C
	ATOM	6635	NZ	LYS	B	218	-9.920	6.379	46.090	1.00	23.12	N
	ATOM	6639	C	LYS	B	218	-9.431	0.985	45.054	1.00	9.00	C
	ATOM	6640	O	LYS	B	218	-8.543	0.568	45.790	1.00	10.38	O
20	ATOM	6641	N	ASN	B	219	-10.709	1.008	45.430	1.00	8.68	N
	ATOM	6643	CA	ASN	B	219	-11.124	0.530	46.752	1.00	8.69	C
	ATOM	6645	CB	ASN	B	219	-12.545	1.004	47.075	1.00	9.62	C
	ATOM	6648	CG	ASN	B	219	-12.589	2.441	47.549	1.00	10.94	C
25	ATOM	6649	OD1	ASN	B	219	-11.678	2.906	48.223	1.00	14.01	O
	ATOM	6650	ND2	ASN	B	219	-13.697	3.138	47.267	1.00	11.68	N
	ATOM	6653	C	ASN	B	219	-11.040	-0.980	46.901	1.00	8.48	C
	ATOM	6654	O	ASN	B	219	-11.108	-1.494	48.016	1.00	9.89	O
	ATOM	6655	N	SER	B	220	-10.884	-1.688	45.792	1.00	8.99	N
	ATOM	6657	CA	SER	B	220	-10.799	-3.141	45.786	1.00	8.99	C
30	ATOM	6659	CB	SER	B	220	-11.517	-3.693	44.555	1.00	9.46	C
	ATOM	6662	OG	SER	B	220	-12.907	-3.416	44.600	1.00	9.93	O
	ATOM	6664	C	SER	B	220	-9.357	-3.642	45.795	1.00	9.37	C
	ATOM	6665	O	SER	B	220	-9.124	-4.844	45.742	1.00	10.44	O
35	ATOM	6666	N	ALA	B	221	-8.377	-2.741	45.851	1.00	9.78	N
	ATOM	6668	CA	ALA	B	221	-6.981	-3.155	45.805	1.00	9.87	C
	ATOM	6670	CB	ALA	B	221	-6.068	-1.948	45.804	1.00	10.44	C
	ATOM	6674	C	ALA	B	221	-6.632	-4.065	46.968	1.00	11.09	C
	ATOM	6675	O	ALA	B	221	-7.064	-3.848	48.094	1.00	12.58	O
	ATOM	6676	N	GLN	B	222	-5.824	-5.080	46.664	1.00	11.56	N
40	ATOM	6678	CA	GLN	B	222	-5.345	-6.085	47.610	1.00	13.48	C
	ATOM	6680	CB	BGLN	B	222	-5.070	-7.420	46.900	0.35	14.51	C
	ATOM	6681	CB	AGLN	B	222	-5.003	-7.403	46.863	0.65	14.16	C
	ATOM	6686	CG	BGLN	B	222	-3.617	-7.830	46.798	0.35	16.11	C
45	ATOM	6687	CG	AGLN	B	222	-6.230	-8.072	46.189	0.65	12.89	C
	ATOM	6692	CD	BGLN	B	222	-3.455	-9.200	46.202	0.35	17.67	C
	ATOM	6693	CD	AGLN	B	222	-5.908	-9.289	45.310	0.65	14.84	C
	ATOM	6694	OE1BGLN	B	222	-4.040	-10.165	46.695	0.35	19.06	O	
	ATOM	6695	OE1AGLN	B	222	-4.806	-9.840	45.371	0.65	18.23	O	
	ATOM	6696	NE2BGLN	B	222	-2.655	-9.300	45.148	0.35	18.44	N	
50	ATOM	6697	NE2AGLN	B	222	-6.880	-9.712	44.495	0.65	13.26	N	
	ATOM	6702	C	GLN	B	222	-4.109	-5.562	48.352	1.00	14.27	C
	ATOM	6703	O	GLN	B	222	-3.636	-6.231	49.284	1.00	17.27	O
	ATOM	6704	OXT	GLN	B	222	-3.579	-4.486	48.029	1.00	15.01	O
55	ATOM	6705	CA	CA	B	301	-0.643	21.256	17.293	1.00	10.41	CA
	ATOM	13398	N	ASP	F	401	-10.088	3.418	14.402	1.00	20.15	N
	ATOM	13400	CA	ASP	F	401	-10.419	4.298	15.551	1.00	19.20	C
	ATOM	13402	CB	ASP	F	401	-11.005	3.471	16.700	1.00	20.61	C
	ATOM	13405	CG	ASP	F	401	-12.475	3.140	16.497	1.00	22.97	C
	ATOM	13406	OD1	ASP	F	401	-13.045	2.395	17.327	1.00	26.18	O
60	ATOM	13407	OD2	ASP	F	401	-13.144	3.572	15.537	1.00	25.29	O
	ATOM	13408	C	ASP	F	401	-9.196	5.076	16.021	1.00	16.65	C
	ATOM	13409	O	ASP	F	401	-9.239	5.713	17.069	1.00	16.48	O
	ATOM	13412	N	ALA	F	402	-8.115	5.032	15.242	1.00	14.63	N
	ATOM	13414	CA	ALA	F	402	-6.897	5.780	15.549	1.00	12.75	C
	ATOM	13416	CB	ALA	F	402	-7.112	7.245	15.277	1.00	12.61	C

	ATOM	13420	C	ALA	F	402	-6.485	5.557	16.999	1.00	11.06	C
	ATOM	13421	O	ALA	F	402	-6.190	6.500	17.738	1.00	10.74	O
	ATOM	13422	N	PHE	F	403	-6.464	4.296	17.429	1.00	10.84	N
5	ATOM	13424	CA	PHE	F	403	-6.076	4.009	18.798	1.00	10.34	C
	ATOM	13426	CB	PHE	F	403	-6.233	2.517	19.116	1.00	11.44	C
	ATOM	13429	CG	PHE	F	403	-7.671	2.025	19.183	1.00	12.38	C
	ATOM	13430	CD1	PHE	F	403	-8.562	2.511	20.119	1.00	14.77	C
	ATOM	13432	CE1	PHE	F	403	-9.880	2.048	20.187	1.00	17.09	C
10	ATOM	13434	CZ	PHE	F	403	-10.309	1.064	19.322	1.00	18.48	C
	ATOM	13436	CE2	PHE	F	403	-9.424	0.544	18.386	1.00	18.39	C
	ATOM	13438	CD2	PHE	F	403	-8.109	1.018	18.324	1.00	16.19	C
	ATOM	13440	C	PHE	F	403	-4.626	4.428	19.018	1.00	10.00	C
	ATOM	13441	O	PHE	F	403	-3.748	4.110	18.209	1.00	12.25	O
15	ATOM	13442	N	GLU	F	404	-4.372	5.130	20.116	1.00	8.64	N
	ATOM	13444	CA	GLU	F	404	-3.025	5.588	20.427	1.00	8.12	C
	ATOM	13446	CB	GLU	F	404	-2.992	7.120	20.524	1.00	7.95	C
	ATOM	13449	CG	GLU	F	404	-3.122	7.705	19.117	1.00	8.08	C
	ATOM	13452	CD	GLU	F	404	-3.043	9.212	19.009	1.00	7.71	C
20	ATOM	13453	OE1	GLU	F	404	-3.129	9.917	20.027	1.00	8.61	O
	ATOM	13454	OE2	GLU	F	404	-2.901	9.672	17.856	1.00	8.80	O
	ATOM	13455	C	GLU	F	404	-2.442	4.854	21.637	1.00	8.22	C
	ATOM	13456	O	GLU	F	404	-2.865	3.708	21.892	1.00	8.82	O
	ATOM	13457	OXT	GLU	F	404	-1.513	5.394	22.258	1.00	8.53	O

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## PATENT CLAIMS

1. A method for constructing a RP-II protease variant, wherein the variant has at least one altered property as compared to a parent RP-II protease, which method comprises:

a) analyzing the three-dimensional structure of the RP-II protease to identify, on the basis of an evaluation of structural considerations, at least one amino acid residue or at least one structural region of the RP-II protease, which is of relevance for altering said property;

b) modifying the DNA of the polynucleotide encoding the parent to construct a polynucleotide encoding a variant RP-II protease, which in comparison to the parent RP-II protease, has been modified by deletion, substitution or insertion of the amino acid residue or structural part identified in i) so as to alter said property;

c) expressing the variant RP-II protease in a suitable host, and

d) testing the resulting RP-II protease variant for said property.

2. A method of producing a BLC like RP-II protease variant, wherein the variant has at least one altered property as compared to a parent BLC like RP-II protease, which method comprises:

a) producing a model structure of the parent BLC like RP-II protease on the three-dimensional structure of BLC,

b) comparing the model three-dimensional structure of the parent BLC like RP-II protease to the BLC structure by superimposing the structures through matching the CA, CB, C, O, and N atoms of the active site residues,

c) identifying on the basis of the comparison in step a) at least one structural part of the parent BLC like RP-II protease, wherein an alteration in said structural part is predicted to result in an altered property;

d) modifying the nucleic acid sequence encoding the parent BLC like RP-II protease to produce a nucleic acid sequence encoding at least one deletion or substitution of one or more amino acids at a position corresponding to said structural part, or at least one insertion of one or more amino acid residues in positions corresponding to said structural part;

e) performing steps c) and d) iteratively N times, where N is an integer with the

value of one or more;

f) preparing the variant resulting from steps a) - e);

g) testing the stability of said variant; and

h) optionally repeating steps a) - g) recursively; and

5 i) selecting a RP-II protease variant having at least one altered property as compared to the parent RP-II protease.

j) expressing the modified nucleic acid sequence in a host cell to produce the variant RP-II protease;

k) isolating the produced protease;

10 l) purifying the isolated protease and

m) recovering the purified RP-II protease variant.

3. The method of claim 2, wherein step (c) identifies amino acid residue positions located at a distance of 10Å or less to the ion-binding site of the RP-II protease parent,  
15 preferably positions located at a distance of 6 Å or less.

4. The method of claim 2, wherein step (c) identifies amino acid residue positions in the RP-II protease parent, the modification of which provides for the removal of the ion binding site by modification of at least one of the positions identified.  
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5. The method of claim 2, wherein step (c) identifies amino acid residue positions in highly mobile regions of the RP-II protease parent.

7. The method of claim 2, wherein step (c) identifies amino acid residue positions in mobile regions of the RP-II protease parent.  
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8. The method of claim 2, wherein step (c) identifies amino acid residue positions in the parent RP-II protease, the modification of which may create at least one disulfide bridge by insertion of or substitution with at least one Cys residue.  
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9. The method of claim 2, wherein steps (c) and (d) provide for constructing a variant of a parent RP-II protease having a modified surface charge distribution by:

c') identifying, on the surface of the parent RP-II protease, at least one charged amino acid residue;

35 d') modifying the charged residue identified in step (a) through deletion or substitution.

tion with an uncharged amino acid residue;

10. The method of claim 2, wherein steps (c) and (d) provide for constructing a variant of a parent RP-II protease having a modified surface charge distribution by:

5 c") identifying, on the surface of the parent RP-II protease, at least one position being occupied by an uncharged amino acid residue;

d") modifying the charge in that position by substituting the uncharged amino acid residue with a charged amino acid residue or by insertion of a charged amino acid residue at the position.

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11. The method of claim 2, wherein steps (c) and (d) provide for constructing a variant of a parent RP-II protease having a modified surface charge distribution by:

c") identifying, on the surface of the parent RP-II protease, at least one charged amino acid residue;

15 d") substituting the charged amino acid residue identified in step (a) with an amino acid residue having an opposite charge.

12. The method of claim 2, wherein step (c) identifies amino acid residue positions in the parent RP-II protease, the modification of which to Pro may create a RP-II protease variant exhibiting improved stability.

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13. The method of claim 2, wherein step (c) identifies amino acid residue positions in the parent RP-II protease at a distance of less than 10Å from the active site residues.

25 14. The method of one or more of claims 2 to 13, wherein N in step (e) is an integer between 1 and 50, 45, 40, 35, 30, 25, 20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2.

15. A RP-II protease variant comprising at least one modification in an amino acid residue in a position located at a distance of 10Å or less to the ion-binding site, preferably positions located at a distance of 6 Å or less.

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16. The variant of claim 15, wherein modifications are made in at least one of the positions: 1, 2, 3, 4, 5, 6, 7, 8, 143, 144, 145, 146, 158, 159, 160, 161, 162, 194, 199, 200, and 201, preferably positions 2, 3, 4, 5, 6, 7, 144, 159, 160, and 161, and especially the modifications D7E and D7Q in BLC (SEQ ID NO: 2), where the positions refer

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to BLC or corresponding positions.

17. The variant of claims 15 or 16, wherein the modification comprises the substitution of a positively charged amino acid residue with a neutral or negatively charged residue, or the substitution of a neutral residue with a negatively charged residue or the deletion of a positively charged or neutral residue.

18. The variant of claim 15, wherein the ion binding site is removed by modification in at least one of the positions corresponding to positions 144 and or 161 of BLC, especially the modifications H144R and/or D161R,K+H144Q,N in BLC (SEQ ID NO:2).

19. A RP-II protease variant comprising at least one modification in an amino acid residue in highly mobile regions in at least one of the positions corresponding to positions 26-31 (26, 27, 28, 29, 30, and 31); 89-91 (89, 90, and 91); 216-221 (216, 217, 218, 219, 220, and 221) of BLC.

20. The variant of claim 19, wherein the parent is BLC and the modification comprises G30A and/or G91A.

21. A RP-II protease variant comprising at least one modification made in mobile regions in at least one of the positions corresponding to positions 51-56, (51, 52, 53, 54, 55, 56), 88-94, (88, 89, 90, 91, 92, 93, 94), 118-122 (118, 119, 120, 121, 122), and 173-183 (173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183) of BLC, preferably the regions 51-56 and 118-122.

22. A RP-II protease variant having at least one disulfide bridge provided by modifying the amino acid residues in positions 128 and 145 in BLC or corresponding positions to Cys, preferably the substitutions S145C and T128C in BLC or corresponding positions.

23. A RP-II protease variant having a modified surface charge distribution in comparison to the parent RP-II protease comprising modifications in at least one of the positions corresponding to positions 7, 17, 95, 109, 143, 174, 209, 216, of BLC, especially the modifications

D7N, S, T  
Y17R, K, H  
Y95R, K, H  
T109R, K, H  
5 Q143R, K, H  
Q174R, K, H  
E209Q, N  
N216R, K, H

in BLC (SEQ ID NO. 1)

10

24. A RP-II protease variant exhibiting improved stability in comparison to the parent RP-II protease comprising at substitution to Pro in at least one of the positions corresponding to positions 18, 115, 185, 269 and 293 in BLC, especially one or more of the substitutions: T60P, S221P, G193P, V194P in BLC (SEQ ID NO. 1).

15

25. A RP-II protease variant comprising modifications in amino acid residues in positions corresponding to positions 1, 8, 22-35 (22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35), 42-58 (42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58), 82-100 (82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100), 129-135  
20 (129, 130, 131, 132, 133, 134, 135), 141-142, 153-156 (153, 154, 155, 156), 158, 161-171 (161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171), 188-193 (188, 189, 190, 191, 192, 193), 195,, 201-207 (201, 202, 203, 204, 205, 206, 207), 210, 213-214, 217 in BLC at a distance of less than 10Å from the active site residues.

25 26. The RP-II protease variant of any of the claims 15 to 25, further comprising at least one of the modifications (i) amino acid residues in positions that form part of an Asn-Gly sequence being modified by deletion or substitution, preferably with Asp, Gln, Ser, Pro, Thr, or Tyr; (ii) amino acid residues in positions that occupied by a Trp being modified by substitution with Phe, Thr, Gln or Gly; (iii) amino acid residues in positions  
30 that are occupied by Glu or Asp being modified by substitution with Ala; (iv) amino acid residues in positions that are in positions that are the 1<sup>st</sup> or 2<sup>nd</sup> position following a position occupied by a Glu or Asp residue being modified by substitution with a Pro; or (v) amino acid residues in positions that are occupied by a Met being modified by deletion or substitution, preferably with Ser or Ala.

35

27. The RP-II protease of any of claims 15 to 26 that is modified in a number of positions ranging from at least one and up to 50 positions, or from 1 to 45 positions, or from 1 to 40 positions, or from 1 to 35 positions, or from 1 to 30 positions, or from 1 to 25 positions, or from 1 to 20 positions, or from 1 to 15 positions, or from 1 to 14 positions, or from 1 to 13 positions, or from 1 to 12 positions, or from 1 to 11 positions, or from 1 to 10 positions, or from 1 to 9 positions, or from 1 to 8 positions, or from 1 to 7 positions, or from 1 to 6 positions, or from 1 to 5 positions, or from 1 to 4 positions, or from 1 to 3 positions, or from 1 to 2 positions, such modifications comprising substitutions, deletions, insertions and combinations thereof in the indicated number of positions.

28. An isolated polynucleotide comprising a nucleic acid sequence, which encodes for a RP-II protease variant defined or produced in any of the preceding claims.

29. The polynucleotide of claim 28, wherein the nucleic acid sequence has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology with the nucleic acid sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or SEQ ID NO:15.

30. An isolated nucleic acid construct comprising a nucleic acid sequence as defined in any of claims 28-29, operably linked to one or more control sequences capable of directing the expression of the polypeptide in a suitable expression host.

31. A recombinant host cell comprising the nucleic acid construct of claim 30.

32. A method for producing the RP-II variant defined or produced in any of claims 1 to 27 the method comprising:

- a) cultivating the recombinant host cell of claim 31 under conditions conducive to the production of the RP-II protease variant; and
- b) recovering the variant.

33. A detergent composition comprising a RP-II protease variant defined or produced in any of claims 1 to 27.

34. Use of a RP-II protease variant defined or produced in any of claims 1 to 27 for

washing or cleaning purposes.

35. Use of a RP-II protease variant defined or produced in any of claims 1 to 27 for processing food.

5

36. Use of a RP-II protease variant defined or produced in any of claims 1 to 27 for processing feed.

37. Use of a RP-II protease variant defined or produced in any of claims 1 to 27 for the treatment of hides.

10

## **ABSTRACT**

The present invention relates to methods for producing variants of a parent RP-II protease and the variants having altered properties as compared to the parent RP-II protease.

5

13 FEB. 2004

## Modtaget

```

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      [ ]      [ ]      [ 7 ]      [ 8 ]      { }
      * 50      56      62 65      77      83 86 90
BLC 46 GHCIYDTSSGSFAGTATVSPGRNGTSYPYGSVKSTRYFIPSGWRS 90
      .      .      .      .
      [ 9 ]      { }      [ ]      [ 11 ]
      * 99 102 106 110 114      126 131
BLC 91 GNTNYDYGAIELSEPIGNTVGYFGYSYTTSSLVGTTVTISGYPGD 135
      .      .      .      .
      [ 12 ]      [13]      [ 14 ]
      142      151 156 +      * 171 177
BLC 136 KTAGTQWQHSGPIAISETYKLQYAMDTYGGQSGSPVFEQSSSRTN 180
      .      .      .      .
      [ 15 ]      [ 16 ]      { }
      182      192      201 208      219
BLC 181 CSGPCSLAVHTNGVYGGSSYNRGTRITKEVFDNLTNWKNSAQ 222

```

\* Active site residue (47, 96, 167)  
+ Calcium coordination residue (3, 5, 161)  
[] Short strand (9-10, 50-51, 56-57, 114-115)  
[ ] Long strand (22-26, 31-36, 41-44, 62-65, 77-83, 99-102, 126-131, 142-151, 156-159, 171-177, 182-192, 201-205)  
{ } Helix (86-90, 106-110, 208-219)

**Fig. 1**

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AC116	.SVIGSDERTRVTDTTAFPPYRAIVHISSS*****IGSCTGWLIGPKTVA	
MIP	.VVIGDDGRTKVANTRVAPYNSIAYITFG*****GSSCTGTLIAPNKIL	
JA96	.VVIGDDGRTKVTNTRVAPYNSIAYITFG*****GSSCTGTLIAPNKIL	
BO32	.VVIGDDGRTKVANTRVAPYNSIAYITFG*****GSSCTGTLIAPNKIL	
	abcdef	
MPR	.SIIGTDERTRISSTTSFPYRATVQLSIKYPNTSSTYGCTGFLVNPNNTVV	
AA513	.VVIGDDGRRQVQNTSFMPFRALTYIEFG**NLTSTWSCSGGVIGTDLVV	
BLC	TAGHCIYDTSSGSFAGTATVSPGRNGTSYPYGSVKSTRYFIPSGWR*SGN	92
CDJ31	TAGHCIYDTASGSFAGTATVSPGRNGSTYPYGSVTSTRYFIPSGYR*SGN	
AC116	TAGHCYVDTASRSFAGTATVSPGRNGSAYPYGSVTSTRYFIPSGWQ*SGN	
	a.	
MIP	TNGHCVYNTASRSYSAKGSVYPGMNDSTAVNGSANMTEFYVPSGYINTGA	
JA96	TNGHCVYNTATRSYSAKGSVYPGMNDSTAVNGSANMTEFYVPSGYINTGA	
BO32	TNGHCVYNTASRSYSAKGSVYPGMNDSTAVNGSANMTEFYVPSGYINTGA	
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AA513	TNAHCV*****EGSVLAGTVVPGMNNNSQWAYGHYRVTQIIYPDQYRNNGA	
BLC	TNYDYGAIELS*****EPIGNTVG YFGYSYT*TSSLVGT TVTISGYPGDK	136
CDJ31	SNYDYGAIELS*****QPIGNTVG YFGYSYT*TSSLVGSSVTIIGYPGDK	
AC116	SNYDYAAIELS*****QPIGNTVG YFGYSYT*ASSLAGAGVTISGYPGDK	
MIP	SQYDFAVIKTD*****TNIGNTVG YRSIRQ**VTNLTGTTIKISGYPGDK	
JA96	SQYDFAVIKTD*****TNIGNTVG YRSIRQ**VTNLTGTTIKISGYPGDK	
BO32	SQYDFAVIKTD*****TNIGNTVG YRSIRQ**VTNLTGTTIKISGYPGDK	
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BLC	T****AGTQWQHSGPIA ISET*YKLQYAMDTYGGQSGSPVFEQSSSR TNC	181
CDJ31	T****SGTQWQMSGNIAVSET*YKLQY AIDTYGGQSGSPVYEASSSR TNC	
AC116	T****TGTQWQMSGTIAVSET*YKLQY AIDTYGGQSGSPVYEKSSSR TNC	
	abcd	
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JA96	MRSTGKVSQWEMSGPVTREDT*NLAYYTIDTFSGNSGSAMLDQ*****	
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CDJ31	SGPCSLAVHTNG**VYGGSSYNRGTRITKEVFDNL TNWKNSAQ	
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BO32	*NQQIVGVHNAG***YSNGTINGGPKATAAFVEFINYAKAQ**	
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Patent- og  
Varemærkestyrelsen

13 FEB. 2004

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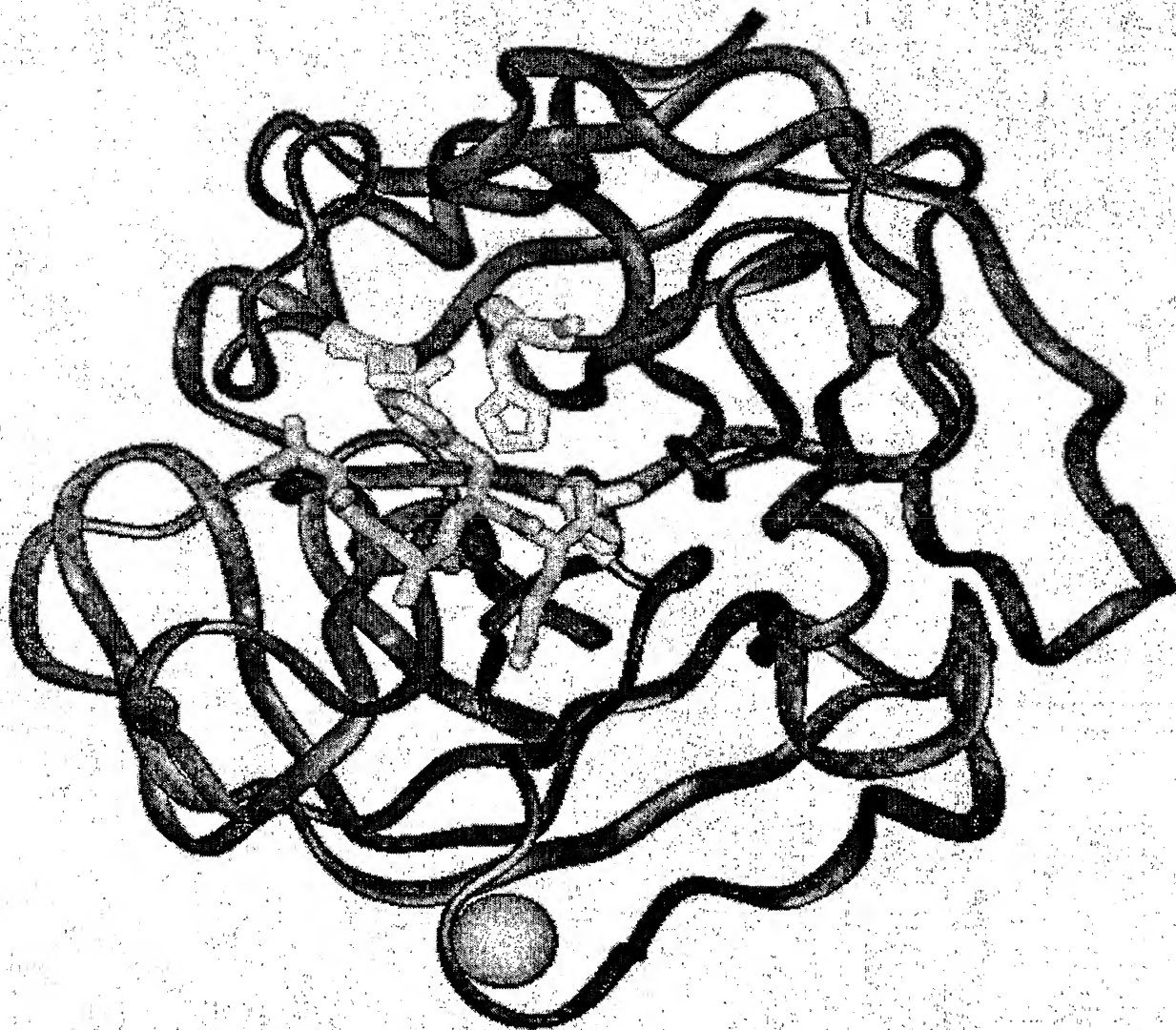


Fig. 3

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Patent- og  
Varemærkestyrelsen

13 FEB. 2004

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 att tct att ttt tct tcg ggc att tac tct gca caa gct gca tca tcg 96  
 Ile Ser Ile Phe Ser Ser Gly Ile Tyr Ser Ala Gln Ala Ala Ser Ser  
 -75 -70 -65  
 ccg cat acc cca gtc tcc agc gac cct tcg tac aag ccc ggc tcc acc 144  
 Pro His Thr Pro Val Ser Ser Asp Pro Ser Tyr Lys Pro Gly Ser Thr  
 -60 -55 -50 -45  
 tat gat ccc aac ata aaa att gac aat aac ggc gca tat tcg aaa gcc 192  
 Tyr Asp Pro Asn Ile Lys Ile Asp Asn Asn Gly Ala Tyr Ser Lys Ala  
 -40 -35 -30  
 ttc gaa gga acc gga aca ccc ggc ggc tcc gtt cag gcc aaa ccg aaa 240  
 Phe Glu Gly Thr Gly Thr Pro Gly Gly Ser Val Gln Ala Lys Pro Lys  
 -25 -20 -15

10517

aaa gaa tcg ccc gcc ggc ccg cct tac agc cct aaa tcg gta atc ggc	288
Lys Glu Ser Pro Ala Gly Pro Pro Tyr Ser Pro Lys Ser Val Ile Gly	
-10 -5 -1 1	
tca gat gaa cgg aca agg gtg act gat aca acg gcc ttt cca tac aga	336
Ser Asp Glu Arg Thr Arg Val Thr Asp Thr Thr Ala Phe Pro Tyr Arg	
5 10 15 20	
gca atc gtc cat att tca agc agc atc ggc tca tgc aca ggc tgg ctg	384
Ala Ile Val His Ile Ser Ser Ser Ile Gly Ser Cys Thr Gly Trp Leu	
25 30 35	
atc gga ccg aaa acg gta gca acg gcc ggg cac tgc gtc tat gac acg	432
Ile Gly Pro Lys Thr Val Ala Thr Ala Gly His Cys Val Tyr Asp Thr	
40 45 50	
gca agc cga tca ttc gcg gga acc gcc acc gtt tcc ccg gga cga aac	480
Ala Ser Arg Ser Phe Ala Gly Thr Ala Thr Val Ser Pro Gly Arg Asn	
55 60 65	
ggg tca gct tac cct tac gga tct gtt aca tcg acc cgc tat ttc atc	528
Gly Ser Ala Tyr Pro Tyr Gly Ser Val Thr Ser Thr Arg Tyr Phe Ile	
70 75 80	
ccg tcg ggt tgg cag agc gga aat tcc aat tat gac tac gca gcg atc	576
Pro Ser Gly Trp Gln Ser Gly Asn Ser Asn Tyr Asp Tyr Ala Ala Ile	
85 90 95 100	
gag ctc agc cag ccg atc ggc aat acc gtc gga tat ttc gga tat tca	624
Glu Leu Ser Gln Pro Ile Gly Asn Thr Val Gly Tyr Phe Gly Tyr Ser	
105 110 115	
tac acc gct tca tcg ctt gca gga gca ggc gtg acc atc agc gga tat	672
Tyr Thr Ala Ser Ser Leu Ala Gly Ala Gly Val Thr Ile Ser Gly Tyr	
120 125 130	
cca gga gac aaa aca aca ggc acc cag tgg caa atg tcc gga acg atc	720
Pro Gly Asp Lys Thr Thr Gly Thr Gln Trp Gln Met Ser Gly Thr Ile	
135 140 145	
gct gtt tca gaa acg tat aaa ctg caa tat gcg atc gac aca tac gga	768
Ala Val Ser Glu Thr Tyr Lys Leu Gln Tyr Ala Ile Asp Thr Tyr Gly	
150 155 160	
ggg caa agc ggt tcc ccg gta tat gag aaa agc agt tca agg aca aac	816
Gly Gln Ser Gly Ser Pro Val Tyr Glu Lys Ser Ser Ser Arg Thr Asn	
165 170 175 180	
tgc agc ggc cca tgc tcg ctg gcc gtt cat acg aac ggc gtg tac gga	864
Cys Ser Gly Pro Cys Ser Leu Ala Val His Thr Asn Gly Val Tyr Gly	
185 190 195	
gga tcc tct tac aac aga ggc acc cgc att acg aaa gaa gta ttt gat	912
Gly Ser Ser Tyr Asn Arg Gly Thr Arg Ile Thr Lys Glu Val Phe Asp	
200 205 210	
aat ttc aca agc tgg aaa aac agc gca cag	942
Asn Phe Thr Ser Trp Lys Asn Ser Ala Gln	
215 220	

<210> 6  
 <211> 314  
 <212> PRT  
 <213> Bacillus licheniformis AC116

<400> 6  
 Met Ala Lys Asn Gly Val Ser Arg Val Phe Ile Ala Gly Leu Ile Gly

10517

-90		-85		-80
Ile Ser -75	Ile Phe Ser Ser -70	Gly Ile Tyr Ser Ala Gln -65	Ala Ala Ser Ser	
Pro His Thr Pro Val Ser -60	Ser Ser Asp Pro Ser Tyr -55	Lys Pro Gly Ser Thr -50	Thr	
Tyr Asp Pro Asn Ile -40	Lys Ile Asp Asn Asn -35	Gly Ala Tyr Ser Lys -30	Ala	
Phe Glu Gly Thr Gly Thr -25	Pro Gly Gly Ser Val Gln -20	Ala Lys Pro Lys		
Lys Glu Ser Pro Ala Gly Pro -10	Pro Tyr Ser Pro Lys Ser -5	Val Ile Gly		
Ser Asp Glu Arg Thr Arg 5	Val Thr Asp Thr Thr 10	Ala Phe Pro Tyr Arg 15		
Ala Ile Val His Ile 25	Ser Ser Ser Ile Gly 30	Ser Cys Thr Gly Trp 35	Leu	
Ile Gly Pro Lys Thr Val 40	Ala Thr Ala Gly His 45	Cys Val Tyr Asp Thr 50		
Ala Ser Arg Ser Phe Ala 55	Gly Thr Ala Thr Val Ser 60	Pro Gly Arg Asn 65		
Gly Ser Ala Tyr Pro Tyr 70	Gly Ser Val Thr Ser Thr 75	Arg Tyr Phe Ile 80		
Pro Ser Gly Trp Gln Ser 85	Gly Asn Ser Asn Tyr 90	Asp Tyr Ala Ala Ile 95		
Glu Leu Ser Gln Pro Ile 105	Gly Asn Thr Val Gly 110	Tyr Phe Gly Tyr Ser 115		
Tyr Thr Ala Ser Ser Leu 120	Ala Gly Ala Gly Val Thr 125	Ile Ser Gly Tyr 130		
Pro Gly Asp Lys Thr Thr 135	Gly Thr Gln Trp Gln Met 140	Ser Gly Thr Ile 145		
Ala Val Ser Glu Thr Tyr 150	Lys Leu Gln Tyr Ala Ile 155	Asp Thr Tyr Gly 160		
Gly Gln Ser Gly Ser Pro 165	Val Tyr Glu Lys Ser 170	Ser Ser Arg Thr Asn 175		
Cys Ser Gly Pro Cys Ser 185	Leu Ala Val His Thr 190	Asn Gly Val Tyr Gly 195		
Gly Ser Ser Tyr Asn Arg 200	Gly Thr Arg Ile Thr 205	Lys Glu Val Phe Asp 210		
Asn Phe Thr Ser Trp Lys 215	Asn Ser Ala Gln 220			

<210> 7  
 <211> 909  
 <212> DNA  
 <213> Bacillus pumilus B032

<220>  
 <221> CDS

&lt;222&gt; (1)..(909)

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (265)..(909)

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; (1)..(78)

&lt;223&gt; pro-peptide (79) ... (264)

&lt;400&gt; 7

atg atg aaa aag gtg aaa atg tta ctc cct tct cta ctt gtt ttt ggt	48
Met Met Lys Lys Val Lys Met Leu Leu Pro Ser Leu Leu Val Phe Gly	
-85 -80 -75	
gct tta agt gtg cct agt ttt gcc cat gcc gca tct gat tca gtg cta	96
Ala Leu Ser Val Pro Ser Phe Ala His Ala Ala Ser Asp Ser Val Leu	
-70 -65 -60	
acg tct gat tat gac atg gtg act tct gat gga aag gtg atc tct tca	144
Thr Ser Asp Tyr Asp Met Val Thr Ser Asp Gly Lys Val Ile Ser Ser	
-55 -50 -45	
agt gat ttc cac aat gat acg aaa tcc ccc tca tcc ttt gat aaa gtg	192
Ser Asp Phe His Asn Asp Thr Lys Ser Pro Ser Phe Asp Lys Val	
-40 -35 -30 -25	
gat gat cta tct tca act gtt ggt gaa aaa gta aaa cca cta tca aaa	240
Asp Asp Leu Ser Ser Thr Val Gly Glu Lys Val Lys Pro Leu Ser Lys	
-20 -15 -10	
tat tta aaa gac ttt caa aca aaa gtc gtc att gga gac gat ggt aga	288
Tyr Leu Lys Asp Phe Gln Thr Lys Val Val Ile Gly Asp Asp Gly Arg	
-5 -1 1 5	
aca aaa gta gca aat aca aga gtg gca cca tat aat tca att gct tat	336
Thr Lys Val Ala Asn Thr Arg Val Ala Pro Tyr Asn Ser Ile Ala Tyr	
10 15 20	
act acg ttt ggc ggc tcc agc tgc acg ggg acc ctg att gcc cct aac	384
Thr Thr Phe Gly Gly Ser Ser Cys Thr Gly Thr Leu Ile Ala Pro Asn	
25 30 35 40	
aaa att ttg aca aac gga cac tgc gtg tac aat aca gca tcc aga agt	432
Lys Ile Leu Thr Asn Gly His Cys Val Tyr Asn Thr Ala Ser Arg Ser	
45 50 55	
tat agt gca aaa gga tgc gtg tat cca ggc atg aat gat agt act gcg	480
Tyr Ser Ala Lys Gly Ser Val Tyr Pro Gly Met Asn Asp Ser Thr Ala	
60 65 70	
gtg aat ggc tca gca aat atg aca gag ttc tat gta cca agc ggg tat	528
Val Asn Gly Ser Ala Asn Met Thr Glu Phe Tyr Val Pro Ser Gly Tyr	
75 80 85	
atc aat aca ggt gcg agc caa tat gat ttt gcc gtg atc aaa aca gat	576
Ile Asn Thr Gly Ala Ser Gln Tyr Asp Phe Ala Val Ile Lys Thr Asp	
90 95 100	
acg aac att ggc aat aca gtt ggt tac cgt tcc atc cgt cag gtg aca	624
Thr Asn Ile Gly Asn Thr Val Gly Tyr Arg Ser Ile Arg Gln Val Thr	
105 110 115 120	
aac tta act ggg aca acg att aaa att tct gga tat cca ggt gat aaa	672
Asn Leu Thr Gly Thr Thr Ile Lys Ile Ser Gly Tyr Pro Gly Asp Lys	
125 130 135	

10517

atg	aga	tca	act	ggc	aag	atc	tcg	cag	tgg	gag	atg	tca	ggg	cct	gtg	720
Met	Arg	Ser	Thr	Gly	Lys	Ile	Ser	Gln	Trp	Glu	Met	Ser	Gly	Pro	Val	
			140					145					150			
aca	aga	gaa	gat	acg	aat	ctc	gca	tac	tat	atg	att	gat	aca	ttt	agt	768
Thr	Arg	Glu	Asp	Thr	Asn	Leu	Ala	Tyr	Tyr	Met	Ile	Asp	Thr	Phe	Ser	
		155					160					165				
gga	aat	tca	ggc	tca	gcg	atg	cta	gat	caa	aat	cag	caa	att	gtt	ggg	816
Gly	Asn	Ser	Gly	Ser	Ala	Met	Leu	Asp	Gln	Asn	Gln	Gln	Ile	Val	Gly	
	170					175					180					
gtt	cat	aac	gca	ggg	tat	tca	aac	ggg	acg	att	aat	ggc	ggg	cca	aaa	864
Val	His	Asn	Ala	Gly	Tyr	Ser	Asn	Gly	Thr	Ile	Asn	Gly	Gly	Pro	Lys	
185					190					195					200	
gcg	aca	gct	gcc	ttt	gtt	gaa	ttt	atc	aac	tat	gca	aaa	gcg	caa		909
Ala	Thr	Ala	Ala	Phe	Val	Glu	Phe	Ile	Asn	Tyr	Ala	Lys	Ala	Gln		
				205					210					215		

<210> 8  
 <211> 303  
 <212> PRT  
 <213> Bacillus pumilus B032

<400> 8  
 Met Met Lys Lys Val Lys Met Leu Leu Pro Ser Leu Leu Val Phe Gly  
                   -85                  -80                  -75

Ala Leu Ser Val Pro Ser Phe Ala His Ala Ala Ser Asp Ser Val Leu  
                   -70                  -65                  -60

Thr Ser Asp Tyr Asp Met Val Thr Ser Asp Gly Lys Val Ile Ser Ser  
                   -55                  -50                  -45

Ser Asp Phe His Asn Asp Thr Lys Ser Pro Ser Ser Phe Asp Lys Val  
                   -40                  -35                  -30                  -25

Asp Asp Leu Ser Ser Thr Val Gly Glu Lys Val Lys Pro Leu Ser Lys  
                   -20                  -15                  -10

Tyr Leu Lys Asp Phe Gln Thr Lys Val Val Ile Gly Asp Asp Gly Arg  
                   -5                  -1                  1                  5

Thr Lys Val Ala Asn Thr Arg Val Ala Pro Tyr Asn Ser Ile Ala Tyr  
                   10                  15                  20

Thr Thr Phe Gly Gly Ser Ser Cys Thr Gly Thr Leu Ile Ala Pro Asn  
                   25                  30                  35                  40

Lys Ile Leu Thr Asn Gly His Cys Val Tyr Asn Thr Ala Ser Arg Ser  
                   45                  50                  55

Tyr Ser Ala Lys Gly Ser Val Tyr Pro Gly Met Asn Asp Ser Thr Ala  
                   60                  65                  70

Val Asn Gly Ser Ala Asn Met Thr Glu Phe Tyr Val Pro Ser Gly Tyr  
                   75                  80                  85

Ile Asn Thr Gly Ala Ser Gln Tyr Asp Phe Ala Val Ile Lys Thr Asp  
                   90                  95                  100

Thr Asn Ile Gly Asn Thr Val Gly Tyr Arg Ser Ile Arg Gln Val Thr  
                   105                  110                  115                  120

Asn Leu Thr Gly Thr Thr Ile Lys Ile Ser Gly Tyr Pro Gly Asp Lys  
                   125                  130                  135

10517

Met Arg Ser Thr Gly Lys Ile Ser Gln Trp Glu Met Ser Gly Pro Val  
 140 145 150  
 Thr Arg Glu Asp Thr Asn Leu Ala Tyr Tyr Met Ile Asp Thr Phe Ser  
 155 160 165  
 Gly Asn Ser Gly Ser Ala Met Leu Asp Gln Asn Gln Gln Ile Val Gly  
 170 175 180  
 Val His Asn Ala Gly Tyr Ser Asn Gly Thr Ile Asn Gly Gly Pro Lys  
 185 190 195 200  
 Ala Thr Ala Ala Phe Val Glu Phe Ile Asn Tyr Ala Lys Ala Gln  
 205 210 215

<210> 9  
 <211> 954  
 <212> DNA  
 <213> Bacillus licheniformis CDJ31

<220>  
 <221> CDS  
 <222> (1)..(954)

<220>  
 <221> mat\_peptide  
 <222> (289)..(954)

<220>  
 <221> sig\_peptide  
 <222> (1)..(84)  
 <223> pro-peptide (85) ... (288)

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 atg aaa aaa agt gtg aca cgc gta tta atg gcc ggt ctt att gga ata 48  
 Met Lys Lys Ser Val Thr Arg Val Leu Met Ala Gly Leu Ile Gly Ile  
 -95 -90 -85  
 tct att tat tct atg ggc atc gac tcc gct caa gct gca tca tcg ccg 96  
 Ser Ile Tyr Ser Met Gly Ile Asp Ser Ala Gln Ala Ala Ser Ser Pro  
 -80 -75 -70 -65  
 cat act cct gtc tct agc gat cct tca tac aag ccc gac tca tcc gca 144  
 His Thr Pro Val Ser Ser Asp Pro Ser Tyr Lys Pro Asp Ser Ser Ala  
 -60 -55 -50  
 agc tat gat cct gct att aaa acc aac aaa aac ggc gcc tat tca aaa 192  
 Ser Tyr Asp Pro Ala Ile Lys Thr Asn Lys Asn Gly Ala Tyr Ser Lys  
 -45 -40 -35  
 gca ttt gaa ggt aca gga aaa cta gac gct ccc ctt tat cag gaa aaa 240  
 Ala Phe Glu Gly Thr Gly Lys Leu Asp Ala Pro Leu Tyr Gln Glu Lys  
 -30 -25 -20  
 agc aaa cca acc aaa aaa tcc cct gcc gga cca cgt tac agc ccc aaa 288  
 Ser Lys Pro Thr Lys Lys Ser Pro Ala Gly Pro Arg Tyr Ser Pro Lys  
 -15 -10 -5 -1  
 tcc gtg att ggt tct gat gaa cgg acg aga gtg aca aac act acc gca 336  
 Ser Val Ile Gly Ser Asp Glu Arg Thr Arg Val Thr Asn Thr Thr Ala  
 1 5 10 15  
 tat cca tac aga gcg atc gtg cat att tca agc agc atc ggg tct tgc 384

10517

Tyr	Pro	Tyr	Arg 20	Ala	Ile	Val	His	Ile 25	Ser	Ser	Ser	Ile	Gly 30	Ser	Cys	
acc	ggc	tcc	ctg	atc	ggt	ccg	aaa	acg	gtg	gca	acg	gcc	gga	cac	tgc	432
Thr	Gly	Ser 35	Leu	Ile	Gly	Pro	Lys 40	Thr	Val	Ala	Thr	Ala 45	Gly	His	Cys	
att	tat	gac	aca	gcg	agc	ggg	tca	ttc	gcc	gga	acc	gct	acc	ggt	tct	480
Ile	Tyr 50	Asp	Thr	Ala	Ser	Gly 55	Ser	Phe	Ala	Gly	Thr 60	Ala	Thr	Val	Ser	
ccg	gga	cgg	aac	ggt	tca	aca	tat	ccg	tac	gga	tca	ggt	aca	tca	acc	528
Pro	Gly	Arg	Asn	Gly	Ser 70	Thr	Tyr	Pro	Tyr	Gly 75	Ser	Val	Thr	Ser	Thr 80	
cgc	tat	ttc	atc	ccg	tca	ggc	tat	cga	agc	gga	aat	tcg	aat	tac	gac	576
Arg	Tyr	Phe	Ile	Pro 85	Ser	Gly	Tyr	Arg	Ser 90	Gly	Asn	Ser	Asn	Tyr 95	Asp	
tac	gga	gcc	ata	gag	ctc	agc	cag	ccg	atc	ggc	aac	acc	gtc	ggg	tat	624
Tyr	Gly	Ala	Ile 100	Glu	Leu	Ser	Gln	Pro 105	Ile	Gly	Asn	Thr	Val 110	Gly	Tyr	
ttc	gga	tat	tcc	tac	acc	acc	tcg	tct	ctc	ggt	ggg	tca	agc	ggt	acc	672
Phe	Gly	Tyr 115	Ser	Tyr	Thr	Thr	Ser 120	Ser	Leu	Val	Gly	Ser 125	Ser	Val	Thr	
atc	atc	gga	tat	cca	ggc	gac	aaa	aca	tcg	ggc	acc	caa	tgg	cag	atg	720
Ile	Ile 130	Gly	Tyr	Pro	Gly	Asp 135	Lys	Thr	Ser	Gly	Thr 140	Gln	Trp	Gln	Met	
tcc	gga	aat	atc	gcc	gtc	tca	gaa	aca	tat	aaa	ctg	caa	tat	gcg	atc	768
Ser	Gly	Asn	Ile	Ala	Val 150	Ser	Glu	Thr	Tyr	Lys 155	Leu	Gln	Tyr	Ala	Ile 160	
gac	aca	tac	gga	ggg	cag	agc	ggc	tct	ccc	gta	tat	gag	gcg	agc	agc	816
Asp	Thr	Tyr	Gly	Gly 165	Gln	Ser	Gly	Ser	Pro 170	Val	Tyr	Glu	Ala	Ser 175	Ser	
tcc	aga	acg	aat	tgc	agc	ggc	cca	tgt	tcg	ctg	gcc	ggt	cat	acg	aat	864
Ser	Arg	Thr	Asn 180	Cys	Ser	Gly	Pro	Cys 185	Ser	Leu	Ala	Val	His 190	Thr	Asn	
ggg	gtg	tac	gga	gga	tct	tca	tac	aac	aga	ggc	acc	cgg	att	aca	aaa	912
Gly	Val	Tyr 195	Gly	Gly	Ser	Ser	Tyr 200	Asn	Arg	Gly	Thr	Arg 205	Ile	Thr	Lys	
gaa	gta	ttc	gat	aat	ttg	aca	aac	tgg	aaa	aac	agc	gcc	caa			954
Glu	Val 210	Phe	Asp	Asn	Leu	Thr 215	Asn	Trp	Lys	Asn	Ser 220	Ala	Gln			

<210> 10  
 <211> 318  
 <212> PRT  
 <213> Bacillus licheniformis CDJ31

<400> 10  
 Met Lys Lys Ser Val Thr Arg Val Leu Met Ala Gly Leu Ile Gly Ile  
 -95 -90 -85  
 Ser Ile Tyr Ser Met Gly Ile Asp Ser Ala Gln Ala Ala Ser Ser Pro  
 -80 -75 -70 -65  
 His Thr Pro Val Ser Ser Asp Pro Ser Tyr Lys Pro Asp Ser Ser Ala  
 -60 -55 -50  
 Ser Tyr Asp Pro Ala Ile Lys Thr Asn Lys Asn Gly Ala Tyr Ser Lys



&lt;223&gt; pro-peptide (76) ... (261)

&lt;400&gt; 11

atg	aaa	aag	gtg	aaa	aaa	tta	atc	cct	tct	cta	ctc	ggt	ttt	ggt	gct	48
Met	Lys	Lys	Val	Lys	Lys	Leu	Ile	Pro	Ser	Leu	Leu	Val	Phe	Gly	Ala	
		-85					-80					-75				
tta	agt	gtg	cct	agt	ttt	gcc	cat	gca	gca	tct	gat	tca	gta	ctt	acg	96
Leu	Ser	Val	Pro	Ser	Phe	Ala	His	Ala	Ala	Ser	Asp	Ser	Val	Leu	Thr	
	-70					-65					-60					
tct	gat	tat	gac	atg	gtg	act	tct	gac	gga	aag	gtg	att	tct	tca	gct	144
Ser	Asp	Tyr	Asp	Met	Val	Thr	Ser	Asp	Gly	Lys	Val	Ile	Ser	Ser	Ala	
-55					-50					-45					-40	
gac	ttc	cac	aac	gat	atg	aaa	acc	ccc	tca	tcc	ttt	gac	aaa	gtg	gat	192
Asp	Phe	His	Asn	Asp	Met	Lys	Thr	Pro	Ser	Ser	Phe	Asp	Lys	Val	Asp	
				-35					-30					-25		
gat	ctc	tct	tct	act	att	ggc	gaa	aaa	gta	aaa	cca	ctc	aca	aca	tat	240
Asp	Leu	Ser	Ser	Thr	Ile	Gly	Glu	Lys	Val	Lys	Pro	Leu	Thr	Thr	Tyr	
			-20				-15						-10			
tta	aaa	gac	ttt	caa	aca	aaa	gta	gtc	att	gga	gac	gat	ggt	aga	aca	288
Leu	Lys	Asp	Phe	Gln	Thr	Lys	Val	Val	Ile	Gly	Asp	Asp	Gly	Arg	Thr	
		-5				-1	1			5						
aaa	gtg	acg	aat	aca	aga	gta	gca	ccc	tat	aat	tct	att	gct	tat	att	336
Lys	Val	Thr	Asn	Thr	Arg	Val	Ala	Pro	Tyr	Asn	Ser	Ile	Ala	Tyr	Ile	
10					15				20					25		
aca	ttt	ggt	gga	tct	agc	tgc	act	gga	aca	ctc	att	gct	cca	aac	aaa	384
Thr	Phe	Gly	Gly	Ser	Ser	Cys	Thr	Gly	Thr	Leu	Ile	Ala	Pro	Asn	Lys	
				30					35					40		
ata	ttg	aca	aac	gga	cac	tgc	gtc	tac	aat	aca	gcc	aca	aga	agt	tat	432
Ile	Leu	Thr	Asn	Gly	His	Cys	Val	Tyr	Asn	Thr	Ala	Thr	Arg	Ser	Tyr	
			45				50						55			
agt	gca	aaa	ggg	tct	gtc	tac	cca	ggc	atg	aat	gac	agc	acg	gct	gtg	480
Ser	Ala	Lys	Gly	Ser	Val	Tyr	Pro	Gly	Met	Asn	Asp	Ser	Thr	Ala	Val	
		60					65				70					
aac	ggc	tca	gca	aac	atg	acc	gaa	ttc	tat	gta	cca	agc	gga	tat	atc	528
Asn	Gly	Ser	Ala	Asn	Met	Thr	Glu	Phe	Tyr	Val	Pro	Ser	Gly	Tyr	Ile	
	75					80					85					
aac	acg	ggg	gcg	agt	caa	tat	gat	ttt	gcc	gtc	att	aaa	aca	gat	acg	576
Asn	Thr	Gly	Ala	Ser	Gln	Tyr	Asp	Phe	Ala	Val	Ile	Lys	Thr	Asp	Thr	
90					95					100					105	
aac	att	gga	aat	acg	gtc	ggc	tat	cgc	tct	att	cgt	caa	gtg	aca	aat	624
Asn	Ile	Gly	Asn	Thr	Val	Gly	Tyr	Arg	Ser	Ile	Arg	Gln	Val	Thr	Asn	
				110				115					120			
cta	aca	ggt	aca	acg	att	aaa	att	tct	gga	tat	cca	ggt	gat	aaa	atg	672
Leu	Thr	Gly	Thr	Thr	Ile	Lys	Ile	Ser	Gly	Tyr	Pro	Gly	Asp	Lys	Met	
			125					130					135			
aga	tcg	act	ggc	aaa	gtg	tca	caa	tgg	gaa	atg	tca	ggt	cca	gtc	acg	720
Arg	Ser	Thr	Gly	Lys	Val	Ser	Gln	Trp	Glu	Met	Ser	Gly	Pro	Val	Thr	
		140					145					150				
aga	gaa	gat	acg	aat	ctc	gca	tac	tat	acg	atc	gat	aca	ttt	agc	gga	768
Arg	Glu	Asp	Thr	Asn	Leu	Ala	Tyr	Tyr	Thr	Ile	Asp	Thr	Phe	Ser	Gly	
	155					160					165					
aac	tct	ggc	tct	gcg	atg	cta	gat	cag	aac	caa	caa	atc	gtc	ggg	gtc	816

Asn Ser Gly Ser Ala Met Leu Asp Gln Asn Gln Ile Val Gly Val  
170 175 180 185

cat aat gcg ggt tat tca aat gga acg atc aac ggt gga cca aaa gcg 864  
His Asn Ala Gly Tyr 190 Ser Asn Gly Thr Ile Asn Gly Gly Pro Lys Ala 200

act gct gcc ttt gtt gaa ttt atc aac tat gcg aag gcg caa 906  
Thr Ala Ala Phe Val Glu Phe Ile Asn Tyr Ala Lys Ala Gln 215

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<212> PRT  
<213> Bacillus pumilus JA96

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Met Lys Lys Val Lys Lys Leu Ile Pro Ser Leu Leu Val Phe Gly Ala  
-85 -80 -75

Leu Ser Val Pro Ser Phe Ala His Ala Ala Ser Asp Ser Val Leu Thr  
-70 -65 -60

Ser Asp Tyr Asp Met Val Thr Ser Asp Gly Lys Val Ile Ser Ser Ala  
-55 -50 -45 -40

Asp Phe His Asn Asp Met Lys Thr Pro Ser Ser Phe Asp Lys Val Asp  
-35 -30 -25

Asp Leu Ser Ser Thr Ile Gly Glu Lys Val Lys Pro Leu Thr Thr Tyr  
-20 -15 -10

Leu Lys Asp Phe Gln Thr Lys Val Val Ile Gly Asp Asp Gly Arg Thr  
-5 -1 1 5

Lys Val Thr Asn Thr Arg Val Ala Pro Tyr Asn Ser Ile Ala Tyr Ile  
10 15 20 25

Thr Phe Gly Gly Ser Ser Cys Thr Gly Thr Leu Ile Ala Pro Asn Lys  
30 35 40

Ile Leu Thr Asn Gly His Cys Val Tyr Asn Thr Ala Thr Arg Ser Tyr  
45 50 55

Ser Ala Lys Gly Ser Val Tyr Pro Gly Met Asn Asp Ser Thr Ala Val  
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Asn Gly Ser Ala Asn Met Thr Glu Phe Tyr Val Pro Ser Gly Tyr Ile  
75 80 85

Asn Thr Gly Ala Ser Gln Tyr Asp Phe Ala Val Ile Lys Thr Asp Thr  
90 95 100 105

Asn Ile Gly Asn Thr Val Gly Tyr Arg Ser Ile Arg Gln Val Thr Asn  
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Leu Thr Gly Thr Thr Ile Lys Ile Ser Gly Tyr Pro Gly Asp Lys Met  
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Arg Ser Thr Gly Lys Val Ser Gln Trp Glu Met Ser Gly Pro Val Thr  
140 145 150

Arg Glu Asp Thr Asn Leu Ala Tyr Tyr Thr Ile Asp Thr Phe Ser Gly  
155 160 165

Asn Ser Gly Ser Ala Met Leu Asp Gln Asn Gln Gln Ile Val Gly Val  
170 175 180 185

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Lys Ala Ala Glu Asn Pro Gln Thr Ser Val Ser Asn Thr Gly Lys Glu  
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Gly Thr Asp Glu Arg Thr Arg Ile Ser Ser Thr Thr Ser Phe Pro Tyr  
5 10 15  
  
aga gca acc gtt caa ctg tca atc aag tat ccc aac act tca agc act 384  
Arg Ala Thr Val Gln Leu Ser Ile Lys Tyr Pro Asn Thr Ser Ser Thr  
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tat gga tgt acc gga ttt tta gtc aat cca aat aca gtc gtc acg gct 432  
Tyr Gly Cys Thr Gly Phe Leu Val Asn Pro Asn Thr Val Val Thr Ala  
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gga cat tgt gtg tac agc cag gat cat gga tgg gct tcg acg ata acc 480  
Gly His Cys Val Tyr Ser Gln Asp His Gly Trp Ala Ser Thr Ile Thr  
55 60 65

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gcc gcg ccg ggc cgc aat ggt tcg tca tat ccg tac ggt act tat tca 528  
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ggc acg atg ttt tac tcc gtc aaa gga tgg acg gaa agc aaa gac acc 576  
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aac tat gat tac gga gct att aaa tta aac ggt tct cct gga aac acg 624  
 Asn Tyr Asp Tyr Gly Ala Ile Lys Leu Asn Gly Ser Pro Gly Asn Thr  
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gtt ggc tgg tac ggc tac cgg act aca aac agc agc agt ccc gtg ggc 672  
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acc tat aca acc gat acg tac ggc tgc caa agc ggc tcg cct gtt tat 816  
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cga aac tac agt gat aca ggg cag aca gct att gcc att cac acg aac 864  
 Arg Asn Tyr Ser Asp Thr Gly Gln Thr Ala Ile Ala Ile His Thr Asn  
 180 185 190 195

gga gga tcg tca tat aac ttg gga aca agg gtg acg aac gat gta ttc 912  
 Gly Gly Ser Ser Tyr Asn Leu Gly Thr Arg Val Thr Asn Asp Val Phe  
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Pro Tyr Glu Gly Thr Gly Lys Thr Ser Lys Ser Leu Tyr Gly Gly Gln  
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 -10 -5 -1 1

Gly Thr Asp Glu Arg Thr Arg Ile Ser Ser Thr Thr Ser Phe Pro Tyr

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 Tyr Gly Cys Thr Gly Phe Leu Val Asn Pro Asn Thr Val Val Thr Ala  
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 Gly His Cys Val Tyr Ser Gln Asp His Gly Trp Ala Ser Thr Ile Thr  
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 Asn Tyr Asp Tyr Gly Ala Ile Lys Leu Asn Gly Ser Pro Gly Asn Thr  
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 Met Trp Ser Asp Thr Lys Pro Ile Arg Ser Ala Glu Thr Tyr Lys Leu  
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gct tta agt gtg cct agt ttt gcc cat gcc aca tcg gat tca gta cta 96  
 Page 18

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Thr	Ser	Asp	Tyr	Asp	Met	Val	Thr	Ser	Asp	Gly	Lys	Val	Ile	Ser	Ser	
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Ser	Asp	Phe	His	Asn	Asp	Thr	Lys	Ser	Pro	Ser	Ser	Phe	Asp	Lys	Val	
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Asp	Asp	Leu	Ser	Ser	Thr	Ser	Gly	Glu	Lys	Val	Lys	Pro	Leu	Ser	Lys	
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Tyr	Leu	Lys	Asp	Phe	Gln	Thr	Lys	Val	Val	Ile	Gly	Asp	Asp	Gly	Arg	
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aca	aaa	gta	gca	aac	aca	aga	gtg	gca	cca	tat	aat	tca	att	gct	tat	336
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Ile	Thr	Phe	Gly	Gly	Ser	Ser	Cys	Thr	Gly	Thr	Leu	Ile	Ala	Pro	Asn	
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Tyr	Ser	Ala	Lys	Gly	Ser	Val	Tyr	Pro	Gly	Met	Asn	Asp	Ser	Thr	Ala	
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gtg	aat	ggc	tca	gca	aac	atg	acg	gag	ttc	tat	gta	cca	agc	gga	tat	528
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Thr	Asn	Ile	Gly	Asn	Thr	Val	Gly	Tyr	Arg	Ser	Ile	Arg	Gln	Val	Thr	
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Thr	Arg	Glu	Asp	Thr	Asn	Leu	Ala	Tyr	Tyr	Thr	Ile	Asp	Thr	Phe	Ser	
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Gly	Asn	Ser	Gly	Ser	Ala	Met	Leu	Asp	Gln	Asn	Gln	Gln	Ile	Val	Gly	
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Val	His	Asn	Ala	Gly	Tyr	Ser	Asn	Gly	Thr	Ile	Asn	Gly	Gly	Pro	Lys	
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Tyr Leu Lys Asp Phe Gln Thr Lys Val Val Ile Gly Asp Asp Gly Arg  
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Lys Ile Leu Thr Asn Gly His Cys Val Tyr Asn Thr Ala Ser Arg Ser  
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Tyr Ser Ala Lys Gly Ser Val Tyr Pro Gly Met Asn Asp Ser Thr Ala  
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Val Asn Gly Ser Ala Asn Met Thr Glu Phe Tyr Val Pro Ser Gly Tyr  
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Ile Asn Thr Gly Ala Ser Gln Tyr Asp Phe Ala Val Ile Lys Thr Asp  
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